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(71) Applicant *(for all designated States except US)*: CEDARS-SINAI MEDICAL CENTER [US/US]; 8700 Beverly Boulevard, Los Angeles, CA 90048-1865 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: PULST, Stefan-M. [DE/DE]; 8125 Skyline Drive, Los Angeles, CA 90046 (US).

(74) Agents: RAMOS, Robert, T. et al.; Campbell & Flores LLP, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).

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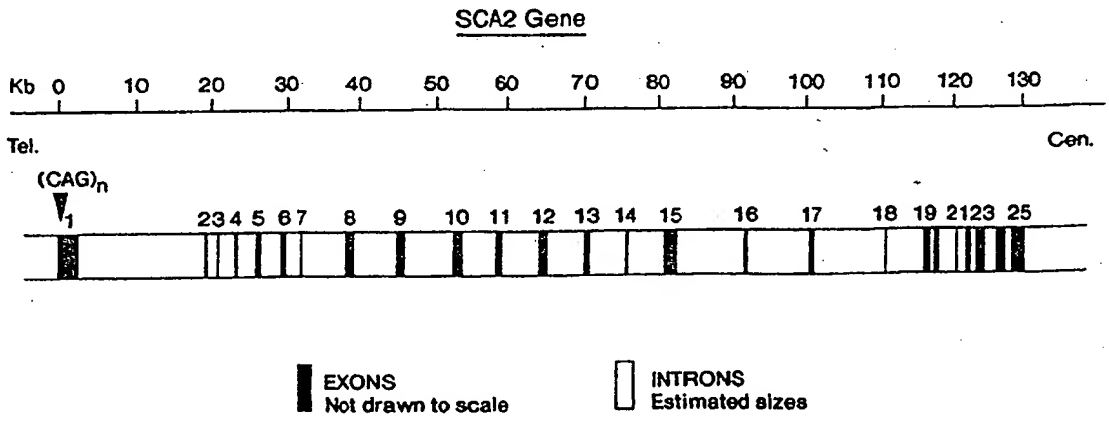

- Largest exon: exon 1, 928 bps; contains CAG repeat
- Largest intron: intron 1 with approximately 15 Kbps
- Smallest exon: exon 2, 37 bps

- Exon sizes:
8<100 bps
100 bps<12<200 bps
200 bps< 4<400 bps
400 bps< 1

- known intron sizes:
intron 2 : 1.6 Kb
intron 19: 0.3 Kb
intron 22: 1.0 Kb
intron 24: 1.6 Kb

(57) Abstract

The present invention provides isolated nucleic acids encoding human SCA2 protein, or fragments thereof, and isolated SCA2 proteins encoded thereby. Further provided are vectors containing invention nucleic acids, probes that hybridize thereto, host cells transformed therewith, antisense oligonucleotides thereto and compositions containing antibodies that specifically bind to invention polypeptides, as well as transgenic non-human mammals that express the invention protein. In addition, methods for diagnosing spinocerebellar Ataxia Type 2 are provided.

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NUCLEIC ACID ENCODING SPINOCEREBELLAR ATAXIA-2 AND PRODUCTS RELATED THERETO

BACKGROUND OF THE INVENTION

Disorders of the cerebellum and its connections are a major cause of neurologic morbidity and mortality. One of the cardinal features of lesions in these pathways is ataxia or incoordination of movements and gait. Although some of the lesions have obvious etiologies such as trauma, strokes or tumors, the etiology of many ataxias has remained difficult to define and is due to metabolic deficiencies, remote effects of cancer or genetic causes. Hereditary spinocerebellar degenerations have a prevalence of 7 - 20 cases per 100,000 (Filla et al., *J. of Neurology* 239(6):351-353 (1992); Polo et al., *Brain* 114 (pt2):855-866 (1991)) which equals the estimates for the prevalence of multiple sclerosis in the United States Based on clinical analysis and genetic inheritance patterns several forms of ataxias are now recognized. Among the genetic causes of ataxic disorders, the autosomal dominant spinocerebellar ataxias (SCAs) have been the most difficult to classify and until recently no clues to their cause existed.

The SCAs are progressive degenerative neurological diseases of the nervous system characterized by a progressive degeneration of neurons of the cerebellar cortex. Degeneration is also seen in the deep cerebellar nuclei, brain stem, and spinal cord. Clinically, affected individuals suffer from severe ataxia and dysarthria, as well as from variable degrees of motor disturbance and neuropathy. The disease usually results in complete disability and eventually in death 10 to 30 years after onset of symptoms. The genes for SCA types 1 and 3 have been identified. Both contain CAG DNA repeats that cause the disease when expanded. However, little is known how CAG repeat expansion and consequent elongation of

polyglutamine tracts translate into neurodegeneration. The identification of the SCA2 gene would provide the opportunity to study this phenomenon in a new protein system.

The significance of identifying ataxia genes goes beyond improved diagnosis for individuals, the possibility of prenatal/presymptomatic diagnosis or better classification of ataxias. Most of the genes associated with repeat expansions in the coding region including the genes for SCA1 and SCA3 are genes that show no homology to known genes. Thus, isolation of these genes will likely point to pathways leading to late-onset neurodegeneration that are novel and may have importance for other neurodegenerative diseases.

For example, it has been suggested that CAG expansion may result in increased transglutamination of proteins, a process that has also been implicated in Alzheimer's disease. The ataxias in particular offer the unique opportunity to study how different genes may either independently or through conjoined action in the same pathway produce relatively similar phenotypes in humans. Therefore, it may be possible to examine the interaction of these genes on age of onset and phenotype, and explain that part of phenotypic variability that is not explained by determining repeat expansion in the mutant allele. Cosmids and YACs have been the main tools for generating contig maps of chromosomal regions and the entire genome, respectively. Recently, novel cloning vectors (reviewed in Ioannou et al., *Nat. Genet.* 6:84-89 (1994)) have been developed that may be more stable than cosmids, while being considerable larger.

Several systems of classification have been proposed for the SCAs based on pathological, clinical or genetic criteria. However, these attempts have been

hampered by the extreme variability of disease onset and clinical features within and between families. Among the dominant ataxias only Machado-Joseph disease (MJD) has been clinically defined as a separate disease based on the prominence of basal ganglia involvement. However, since phenotypic variability is remarkable in MJD pedigrees, the assignment of individual cases or small families to this category is difficult. Indeed, after identification of the MJD locus (SCA3) it has become apparent that families with a phenotype not typical of MJD, but resembling SCAs are linked to the same locus as SCA3 families.

The advent of genetic linkage analysis provided a novel means to approach classification of the SCAs. Since the late 70's it was recognized that some SCA pedigrees appeared to show linkage to the HLA locus on CHR6, while others did not. Later this locus, now called SCA1, was further defined using RFLP and microsatellite markers and was mapped centromeric to the HLA locus. After the establishment of flanking markers for the SCA1 gene it became rapidly apparent that many- if not the majority- of SCA families did not show linkage to the SCA1 locus. Recently, a second SCA locus was identified on CHR12 using a large pedigree of Cuban descent (Gispert et al., *Nat. Genet.* 4:295-299 (1993)) and in a pedigree of Southern Italian origin (Pulst et al., *Nat. Genet.* 5:8-10 (1993)). At the same time a third locus for Machado-Joseph disease and other pedigrees with an SCA phenotype was identified on CHR14 (Takiyama et al, *Nat. Genet.* 4:300-304 (1993)). Recently, SCA4 was mapped to CHR16 and SCA5 to CHR11 (Ranum et al., *Nat. Genet.* 8:N3:280-284 (1994)).

Two of the SCA genes have been identified, one by a positional cloning approach, the other by a cDNA based approach. The SCA1 gene was identified by screening a cosmid contig covering the region between the two flanking

markers D6S274 and D6S89 for cosmids containing CAG repeats. A CAG repeat was isolated, and shown to be expanded in affected individuals (Orr et al., *Nat. Genet.* 4:221-226 (1993); see Table 1). The number of CAG repeats are inversely correlated with the age of onset. Recently, the complete coding sequence for the SCA1 gene has been determined. The gene does not appear to be homologous to other known genes. Despite the tissue specific effects of the mutation, SCA1 transcripts are ubiquitously expressed. By RT-PCR analysis, normal and mutated transcripts are found in tissues indicating that repeat expansion does not interfere with transcription.

The SCA3 or MJD gene was identified after several CAG containing cDNA clones had been isolated from a brain cDNA library (Kawaguchi et al., *Nat. Genet.* 8:221-227 (1994)). One of these mapped to CHR 14q32.1, the region previously identified by genetic linkage analysis to contain the SCA3 gene. The CAG repeat was expanded in affected individuals, but appears to show greater meiotic stability than other CAG repeats. The SCA3 gene has no homology to other known genes or motif structures, but related sequences were identified on CHR 8q23, 14q21, and Xp22.1.

Although not an SCA gene in the strict sense, CAG expansion in the gene causing dentatorubral-pallidoluysian atrophy (DRPLA) may also lead to degeneration of cerebellar neurons. This gene was identified by searching published brain cDNA sequences for the presence of CAG repeats. A cDNA mapped to CHR12p was found to harbor a CAG repeat which was expanded in DRPLA patients (Koide et al., *Nat. Genet.* 6:9-13 (1994); Nagafuchi et al., *Nat. Genet.* 6:14-18 (1994)). The gene which has no known homologies is ubiquitously expressed. SCA families linked to markers on CHR 12 have been described in several ethnic backgrounds.

The largest ones are of Cuban ancestry (H pedigree), French-Canadian and Austrian ancestry (SAK and GK pedigrees, Lopes-Cendes et al., *Am. J. Hum. Genet.* **54**:774-781 (1994)) and Italian descent (FS pedigree, Pulst et al., (1993)). A smaller Tunisian pedigree has been described as well (Belal et al., *Neurology* **44**:1423-1426 (1994)). Although all pedigrees have cases with early onset in recent generations, a formal age of onset analysis has only been performed for the FS pedigree. This analysis indicated clear evidence of anticipation (Pulst et al., (1993)).

The phenomenon of unstable DNA repeats raises many fascinating issues. For example, in 1991, La Spada et al. identified a polymorphic CAG repeat in the androgen receptor gene on the X chromosome that was greatly expanded in individuals with spinobulbar muscular atrophy (SBMA, Kennedy syndrome). In short succession, a total of ten diseases were found to be caused by trinucleotide repeat (TNR) expansion (Table 1). Although several unifying concepts emerge from the comparison of diseases caused by TNR expansion, important differences can be recognized as well.

Common to all diseases is a highly polymorphic number of repeats on normal chromosomes. If the repeat number reaches allele sizes in between normal and disease alleles -termed premutations- the repeat becomes unstable and may expand to the size associated with the disease state. Large number repeats have the tendency to expand further, although decreases in size are occasionally seen (Bruner et al., *New Engl. J. Med.* **328**:476-480 (1993); reviewed in Brook, *Nat. Genet.* **3**:279-152 (1993); Mandel, *Nat. Genet.* **4**:8-9 (1993)).

TABLE 1:

Characteristics of diseases caused by TNR expansion

Disease	Type of of repeat	Location of of repeat	Number of repeats in normal alleles	in disease alleles
Fragile X syndrome	CGG	5' untr.	5 - 54	200 - 200
FRAXE	GCC	unknown	6 - 25	200 - 80
FRAXF	GCC	unknown	6 - 29	300 - 500
FRA16A	GCC	unknown	16 - 49	1000 - 20000
Myotonic dystrophy	CTG	3' untr.	5 - 35	100 - 200
SBMA	CAG	coding	11 - 31	40 - 62
Huntington disease	CAG	coding	15 - 38	38 - 120
CA 1	CAG	coding	25 - 36	43 - 81
DRPLA	CAG	coding	7 - 26	49 - 75
MJD (SCA3)	CAG	coding	13 - 36	68 - 79

TNR expansion may be a common form of human mutagenesis. Especially if expansion is not restricted to pure CAG and CCG repeats, the number of genes predisposed to expansion may be quite large. Three diseases with cerebellar degeneration, SCA1, DRPLA, and SCA3 are caused by expansion of a CAG repeat. In these diseases clear evidence of anticipation was lacking, although very early onset cases in some families had raised this question. However, as described in Pulst et al. (1993) strong evidence for anticipation was identified in the FS pedigree with SCA2. Thus, there is a need in the art to identify the location and nucleic acid structure of the SCA2 gene.

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acids encoding the human SCA2 protein and isolated proteins encoded thereby. Further provided are vectors containing invention nucleic acids, probes that hybridize thereto, host cells transformed therewith, antisense oligonucleotides thereto and compositions containing, antibodies that specifically bind to invention polypeptides and compositions containing, as well as transgenic non-human mammals that express the invention protein. In addition, methods for diagnosing

spinocerebellar Ataxia Type 2, or a presisposition thereto, are provided.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a physical map of the SCA2 region. The location of *D12S1328* centromeric and *D12S1329* telomeric of the contig are indicated. As indicated by double forward slashes, the map is not drawn to scale between *D12S1328* and *P46F2t7*, and between *B78E14t7* and *D12S1329*. YAC, PAC and BAC clones are prefixed with 'Y', 'P', and 'B' respectively. Clones positive for a specific STS by PCR analysis are indicated by vertical lines. Solid arrows indicate end-STSs from the clone under the symbol. Sizes of all clones are shown to scale. The chimeric part of YAC clone 856_h_2(1,100 kb) is indicated by a dashed arrow. Interstitial deletions in YACs or PACs are indicated by thin lines in brackets. The extent of the deletion in YAC Y638_e_7 is not precisely known.

Figure 2 shows the nucleic acid sequence (SEQ ID NO:1) of plasmid PL65I22B for genomic DNA encoding the expansion of the CAG repeat in individuals with SCA2. Nucleotides 1 - 499 of Figure 2 correspond to cDNA nucleotides 392 - 890 of Figure 6 (SEQ ID NO:2). The locations of primers SCA2-A and SCA2-B are indicated by arrows. The location of a predicted splice site is indicated by a vertical arrow between nucleotides 499 and 500 (also compare with Figure 6).

Figure 3 shows an analysis of the SCA2 CAG repeat by polyacrylamide electrophoresis. A common allele of 22 repeats and a less frequent allele of 23 repeats (samples 14 and 15) are seen in normal individuals. SCA2 patients with extended alleles form 37

to 52 repeats are shown. SCA2 patients derive from two pedigrees with CHR 12 linked dominant ataxia. The pedigree structures are shown at the top. Genomic DNAs were amplified with primers SCA2-A and SCA2-B and separated in a 6% polyacrylamide gel. Primer SCA2-A was end-labeled. As a size standard, single stranded M13mp18 control DNA was sequenced with sequencing primer "-40" provided by USB (United States Biochem.).

Figure 4 shows a Scattergram indicating that CAG repeat length and age-of-onset of disease in 33 SCA2 patients are inversely correlated.

Figure 5 shows four cDNA clones as a schematic of the composite SCA2 cDNA sequence. The thick line corresponds to coding sequence, the thin line to untranslated regions. The location of the CAG repeat is indicated by a hatched box. In clone S2, the repeat was not a CAG, but a CTG repeat followed by 12 bp of sequence not contained in any of the other cDNA clones.

Figure 6 shows the composite cDNA sequence (SEQ ID NO:2) obtained from assembly of the partially overlapping cDNA clones shown in Figure 5. The predicted SCA2 protein product (SEQ ID NO:3) is shown below the DNA sequence. The stop codon for the SCA2 cDNA is indicated by *. The locations of primers SCA2-A, SCA2-B, and SCA2-B14 are indicated by horizontal arrows. The splice site between primers SCA2-B and SCA2-B14 is indicated by a vertical arrow.

Figure 7 shows a partial amino acid sequence alignment comparison of ataxin-2 protein, the ataxin-2 related protein (A2RP), and the mouse SCA2 homologue in the region of strongest homology. Codon 1 corresponds to codon 155 in Figure 6 (SEQ ID NO:3).

Figure 8 shows the genomic structure of the SCA2 gene.

DETAILED DESCRIPTION OF THE INVENTION

The hereditary ataxias are a complex group of neurodegenerative disorders all characterized by varying abnormalities of balance attributed to dysfunction or pathology of the cerebellum and cerebellar pathways. In many of these disorders, dysfunction or structural abnormalities extend beyond the cerebellum, and may involve basal ganglia function, oculo-motor disorders and neuropathy. Among the inherited ataxias, the classification of dominant adult onset ataxias is particularly controversial with regard to nomenclature, associated findings and pathology. The dominant spinocerebellar ataxias (SCAs) represent a phenotypically heterogeneous group of disorders with a prevalence of familial cases of approximately 1 per 100,000. This group of disorders is also designated as olivo-ponto-cerebellar atrophies (OPCAs), although this term is too restrictive a pathological label.

The high phenotypic variability within single SCA pedigrees has made clinical classification of different forms of ataxia difficult. The gene causing SCA1 has been identified on CHR 6p and the SCA3 gene has been identified on CHR 14q. These diseases are caused by expansion of a CAG repeat in the coding region of the genes. However, many SCA pedigrees do not show linkage to CHR 6p or CHR 14q, confirming the presence of non-allelic heterogeneity. Subsequent genetic linkage studies have led to the identification of SCA loci on CHR12 and some families do not show linkage to either of the above three chromosomal regions.

Described in the instant specification is the construction of the BAC (Bacterial Artificial Chromosome) Shizuya et al., *Proc. Natl. Acad. Sci. USA* 89:8794-8797 (1992) contig and PAC (P1 Artificial Chromosome) of the SCA2 region and the isolation of a novel SCA2 gene from this contiguous map unit using a technique that screens for the presence of DNA trinucleotide repeats.

Sequence analysis of the DNA sequence flanking the CAG repeat revealed an open reading frame of 317 base pairs (Figure 2). A homology search of the amino acid sequence of this open reading frame (ORF) with genes registered in Genbank/EMBL and search of the TIGR database showed no homologous proteins or homologous genomic DNA sequences. Using reverse-transcribed PCR (polymerase chain reaction) with primers SCA1-A and SCA1-B, the genomic sequence containing the CAG repeat was shown to be expressed into mRNA. Subsequently, cDNA encoding human and mouse SCA2 has been isolated as described hereinafter in Examples 4 and 7, respectively.

Accordingly, the present invention provides isolated nucleic acids, which encode a novel mammalian SCA2 protein, and fragments thereof. Such nucleic acids can be obtained, for example, from human chromosome 12, specifically at the q24.1 locus, which is the site of mutation(s) that cause SCA2.

The term "nucleic acids" (also referred to as polynucleotides) encompasses RNA as well as single and double-stranded DNA and cDNA. As used herein, the phrase "isolated" means a nucleic acid that is in a form that does not occur in nature. One means of isolating a nucleic acid encoding an SCA2 polypeptide is to probe a mammalian genomic library with a natural or artificially designed DNA probe using methods well known in the art. DNA probes derived from the SCA2 gene are particularly

useful for this purpose. DNA and cDNA molecules that encode SCA2 polypeptides can be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian (e.g., mouse, rat, rabbit, pig, and the like), or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Examples of nucleic acids are RNA, cDNA, or isolated genomic DNA encoding an SCA2 polypeptide. Such invention nucleic acids may include, but are not limited to, nucleic acids having substantially the same nucleotide sequence as nucleotides 163-4098 set forth in SEQ ID NO:2 (Figure 6), or at least nucleotides 163-657 or nucleotides 724-4098 of SEQ ID NO:2; or nucleotides 50-3454 of SEQ ID NO:4. In a preferred embodiment, invention nucleic acids include the same nucleotide sequence as nucleotides 163-4098 of SEQ ID NO:2, or include the same nucleotide sequence as nucleotides 50-3454 of SEQ ID NO:4.

As employed herein, the phrase "substantially the same nucleotide sequence" refers to DNA having sufficient homology to the reference polynucleotide, such that it will hybridize to the reference nucleotide under typical moderate stringency conditions. In one embodiment, nucleic acid molecules having substantially the same nucleotide sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that of either SEQ ID NO:3, or SEQ ID NO:5. In another embodiment, DNA having "substantially the same nucleotide sequence" as the reference nucleotide sequence has at least 60% homology with respect to the reference nucleotide sequence. DNA having at least 70%, more preferably 80%, yet more preferably 90%, homology to the reference nucleotide sequence is preferred.

This invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:4, but which have the same phenotype. Phenotypically similar nucleic acids are also referred to as "functionally equivalent nucleic acids". As used herein, the phrase "functionally equivalent nucleic acids" encompasses nucleic acids characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acids disclosed herein. In particular, functionally equivalent nucleic acids encode polypeptides that are the same as those disclosed herein or that have conservative amino acid variations. For example, conservative variations include substitution of a non-polar residue with another non-polar residue, or substitution of a charged residue with a similarly charged residue. These variations include those recognized by skilled artisans as those that do not substantially alter the tertiary structure of the protein.

Further provided are nucleic acids encoding SCA2 polypeptides that, by virtue of the degeneracy of the genetic code, do not necessarily hybridize to the invention nucleic acids under specified hybridization conditions. Preferred nucleic acids encoding the invention polypeptide are comprised of nucleotides that encode substantially the same amino acid sequence set forth in SEQ ID NO:3 (Figure 6), or SEQ ID NO:5.

As employed herein, the term "substantially the same amino acid sequence" refers to amino acid sequences having at least about 70% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological properties characteristic of the protein defined by the reference amino acid sequence. Preferably, proteins having "substantially the same amino

acid sequence" will have at least about 80%, more preferably 90% amino acid identity with respect to the reference amino acid sequence (SEQ ID NO:3 or SEQ ID NO:5); with greater than about 95% amino acid sequence identity being especially preferred.

Alternatively, preferred nucleic acids encoding the invention polypeptide(s) hybridize under moderately stringent, preferably high stringency, conditions to substantially the entire sequence, or substantial portions (i.e., typically at least 15-30 nucleotides) of the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2 (Figure 6) or SEQ ID NO:4.

Stringency of hybridization, as used herein, refers to conditions under which polynucleotide hybrids are stable. As known to those of skill in the art, the stability of hybrids is a function of sodium ion concentration and temperature (See, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual 2d Ed.* (Cold Spring Harbor Laboratory, (1989); incorporated herein by reference). Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art.

As used herein, the phrase "moderately stringent" hybridization refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60%, preferably about 75%, more preferably about 85%, homology (i.e., identity) to the target DNA; with greater than about 90% homology to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring

Harbor Laboratory Press, (1989)) are well known to those of skill in the art as are other suitable hybridization buffers.

Also provided are isolated SCA2 peptides, polypeptides(s) and/or protein(s), or fragments thereof, encoded by the invention nucleic acids.

As used herein, the term "isolated" means a protein molecule free of cellular components and/or contaminants normally associated with a native *in vivo* environment. Invention polypeptides and/or proteins include any isolated natural occurring allelic variant, as well as recombinant forms thereof. The SCA2 polypeptides can be isolated using various methods well known to a person of skill in the art. The methods available for the isolation and purification of invention proteins include, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography. Other well-known methods are described in Deutscher et al., *Guide to Protein Purification: Methods in Enzymology* Vol. 182, (Academic Press, (1990)), which is incorporated herein by reference. Alternatively, the isolated polypeptides of the present invention can be obtained using well-known recombinant methods as described, for example, in Sambrook et al., *supra.*, 1989).

An example of the means for preparing the invention polypeptide(s) is to express nucleic acids encoding the SCA2 in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell (i.e., oocyte), or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using well-known methods. Invention polypeptides can be isolated directly from cells that have been transformed

with expression vectors, described below in more detail. The invention polypeptide, biologically active fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.

As used herein, the phrase "SCA2" refers to substantially pure native SCA2 protein, or recombinantly expressed/produced (i.e., isolated or substantially pure) proteins, including variants thereof encoded by mRNA generated by alternative splicing of a primary transcript, and further including fragments thereof which retain native biological activity. Preferred invention polypeptides are those that contain substantially the same amino acid sequence set forth in SEQ ID NO:3 (Figure 6), or at least amino acids 1-165 or amino acids 188-1312 of SEQ ID NO:3, or include substantially the same amino acid sequence set forth in SEQ ID NO:5. As used herein, the phrase "functional polypeptide" means a SCA2 that can produce an anti-SCA2 antibody that binds to the native SCA2 protein or to the amino acid sequence set forth in SEQ ID NO:3 (Figure 6), or SEQ ID NO:5. In a preferred embodiment, invention polypeptides include the same amino acid sequence as set forth in SEQ ID NO:3 or SEQ ID NO:5.

Modification of the invention nucleic acids, polypeptides or proteins with the following phrases: "recombinantly expressed/produced", "isolated", or "substantially pure", encompasses nucleic acids, peptides, polypeptides or proteins that have been produced in such form by the hand of man, and are thus separated from their native *in vivo* cellular environment. As a result of this human intervention, the recombinant nucleic acids, polypeptides and proteins of the invention

are useful in ways that the corresponding naturally occurring molecules are not, such as identification of selective drugs or compounds.

Sequences having "substantially the same sequence" homology are intended to refer to nucleotide sequences that share at least about 75%, preferably about 80%, yet more preferably about 90% identity with invention nucleic acids; and amino acid sequences that typically share at least about 75%, preferably about 85%, yet more preferably about 95% amino acid identity with invention polypeptides. It is recognized, however, that polypeptides or nucleic acids containing less than the above-described levels of homology arising as splice variants or that are modified by conservative amino acid substitutions, or by substitution of degenerate codons are also encompassed within the scope of the present invention.

The present invention provides the isolated polynucleotide encoding SCA2 operatively linked to a promoter of RNA transcription, as well as other regulatory sequences. As used herein, the phrase "operatively linked" refers to the functional relationship of the polynucleotide with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of a polynucleotide to a promoter refers to the physical and functional relationship between the polynucleotide and the promoter such that transcription of DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and wherein the promoter directs the transcription of RNA from the polynucleotide.

Promoter regions include specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. Additionally, promoter regions include sequences that modulate the recognition, binding and transcription initiation activity of RNA polymerase. Such sequences may be *cis* acting or may be responsive to *trans* acting factors. Depending upon the nature of the regulation, promoters may be constitutive or regulated. Examples of promoters are SP6, T4, T7, SV40 early promoter, cytomegalovirus (CMV) promoter, mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia virus (MMLV) promoter, and the like.

Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, *J. Biol. Chem.* 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the SCA2 polypeptide in order to enhance transcription (e.g., the codon preference of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Also provided are vectors comprising invention nucleic acids. Examples of vectors are viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA.

Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Other means are well known and available in the art.

Further provided are vectors comprising nucleic acids encoding SCA2 polypeptides, adapted for expression in a bacterial cell, a yeast cell, an amphibian cell (i.e., oocyte), a mammalian cell and other animal cells. The vectors additionally comprise the regulatory elements

necessary for expression of the nucleic acid in the bacterial, yeast, amphibian, mammalian or animal cells so located relative to the nucleic acid encoding SCA2 polypeptide as to permit expression thereof.

As used herein, "expression" refers to the process by which nucleic acids are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the nucleic acid is derived from genomic DNA, expression may include splicing of the mRNA, if an appropriate eucaryotic host is selected. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Sambrook et al. *supra*). Similarly, a eucaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors can be obtained commercially or assembled by the sequences described in methods well known in the art, for example, the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the invention polypeptide.

The present invention provides transformed host cells that recombinantly express SCA2 polypeptides. An example of a transformed host cell is a mammalian cell comprising a plasmid adapted for expression in a mammalian cell. The plasmid contains nucleic acid encoding an SCA2 polypeptide and the regulatory elements necessary for expression of invention proteins. Various mammalian cells may be utilized as hosts, including, for example, mouse fibroblast cell NIH3T3, CHO cells, HeLa

cells, Ltk- cells, etc. Expression plasmids such as those described *supra* can be used to transfect mammalian cells by methods well known in the art such as, for example, calcium phosphate precipitation, DEAE-dextran, electroporation, microinjection or lipofection.

The present invention provides nucleic acid probes comprising nucleotide sequences capable of specifically hybridizing with sequences included within nucleic acids encoding SCA2 polypeptides, for example, a coding sequence included within the nucleotide sequence shown in SEQ ID NO:2 (Figure 6), or SEQ ID NO:4. In a preferred embodiment, the probe is derived from the nucleic acid sequence set forth in SEQ ID NO:2, or at least nucleotides 163-657 or nucleotides 724-4098 of SEQ ID NO:2; or SEQ ID NO:4. Preferred regions from which to construct probes include 5' and/or 3' coding sequences, sequences within the ORF, and the like. Full-length or fragments of cDNA clones encoding SCA2 can also be used as probes for the detection and isolation of related genes. As used herein, an invention "probe" or invention oligonucleotide is a single-stranded DNA or RNA that has a sequence of nucleotides that includes at least about 15 contiguous bases up to the full length coding region of SEQ ID NO:2 or SEQ ID NO:4. Preferably an invention probe is at least about 30 contiguous bases, more preferably at least about 50, yet more preferably at least about 100, with about 300 contiguous bases up to the full length coding region of SEQ ID NO:2 and SEQ ID NO:4 being especially preferred. When fragments are used as probes, preferably the cDNA sequences will be from the carboxyl end-encoding portion of the cDNA, and most preferably will include predicted transmembrane domain-encoding portions of the cDNA sequence. Transmembrane domain regions can be predicted based on hydropathy analysis of the deduced amino acid sequence using, for

example, the method of Kyte and Doolittle, *J. Mol. Biol.* 157:105 (1982).

As used herein, the phrase "specifically hybridizing" encompasses the ability of a polynucleotide to recognize a sequence of nucleic acids that are complementary thereto and to form double-helical segments via hydrogen bonding between complementary base pairs. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable agent, such as a radioisotope, a fluorescent dye, and the like, to facilitate detection of the probe. Invention probes are useful to detect the presence of nucleic acids encoding the SCA2 polypeptide. For example, the probes can be used for *in situ* hybridizations in order to locate biological tissues in which the invention gene is expressed. Additionally, synthesized oligonucleotides complementary to the nucleic acids of a nucleotide sequence encoding SCA2 polypeptide are useful as probes for detecting the invention genes, their associated mRNA, or for the isolation of related genes using homology screening of genomic or cDNA libraries, or by using amplification techniques well known to one of skill in the art.

Also provided are antisense oligonucleotides having a sequence capable of binding specifically with any portion of an mRNA that encodes SCA2 polypeptides so as to prevent or inhibit translation of the mRNA. The antisense oligonucleotide may have a sequence capable of binding specifically with any portion of the sequence of the cDNA encoding SCA2 polypeptides. As used herein, the phrase "binding specifically" encompasses the ability of a nucleic acid sequence to recognize a complementary nucleic acid sequence and to form double-helical segments therewith via the formation of hydrogen bonds between the

complementary base pairs. An example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogs of nucleotides.

Compositions comprising an amount of the antisense oligonucleotide, described above, effective to reduce expression of SCA2 polypeptides by passing through a cell membrane and binding specifically with mRNA encoding SCA2 polypeptides so as to prevent translation and an acceptable hydrophobic carrier capable of passing through a cell membrane are also provided herein. The acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind to a cell-type specific receptor.

Antisense oligonucleotide compositions are useful to inhibit translation of mRNA encoding invention polypeptides. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding SCA2 polypeptides and inhibit translation of mRNA and are useful as compositions to inhibit expression of SCA2 associated genes in a tissue sample or in a subject.

In accordance with another embodiment of the invention, kits for detecting mutations and aneuploidies in chromosome 12 at locus q24.1 comprising at least one invention probe or antisense nucleotide.

The present invention provides means to modulate levels of expression of SCA2 polypeptides by employing synthetic antisense oligonucleotide compositions (hereinafter SAOC) which inhibit translation of mRNA encoding these polypeptides. Synthetic

oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the SCA2 coding strand or nucleotide sequences shown in SEQ ID NO:2, or SEQ ID NO:4. The SAOC is designed to be stable in the blood stream for administration to a subject by injection, or in laboratory cell culture conditions. The SAOC is designed to be capable of passing through the cell membrane in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOC which render it capable of passing through cell membranes, for example, by designing small, hydrophobic SAOC chemical structures, or by virtue of specific transport systems in the cell which recognize and transport the SAOC into the cell. In addition, the SAOC can be designed for administration only to certain selected cell populations by targeting the SAOC to be recognized by specific cellular uptake mechanisms which bind and take up the SAOC only within select cell populations.

For example, the SAOC may be designed to bind to a receptor found only in a certain cell type, as discussed *supra*. The SAOC is also designed to recognize and selectively bind to target mRNA sequence, which may correspond to a sequence contained within the sequence shown in SEQ ID NO:2, or SEQ ID NO:4. The SAOC is designed to inactivate target mRNA sequence by either binding thereto and inducing degradation of the mRNA by, for example, RNase I digestion, or inhibiting translation of mRNA target sequence by interfering with the binding of translation-regulating factors or ribosomes, or inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups which either degrade or chemically modify the target mRNA. SAOCs have been shown to be capable of such properties when directed against mRNA targets (see Cohen et al., *TIPS*, 10:435

(1989) and Weintraub, *Sci. American*, January (1990), pp.40; both incorporated herein by reference).

The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified SCA2 polypeptide, an active fragment thereof, or a purified, mature protein and active fragments thereof, alone or in combination with each other. These polypeptides or proteins can be recombinantly derived, chemically synthesized or purified from native sources. As used herein, the term "acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

Further provided are anti-SCA2 antibodies having specific reactivity with SCA2 polypeptides of the present invention. Active fragments of antibodies are encompassed within the definition of "antibody". Invention antibodies can be produced by methods known in the art using invention polypeptides, proteins or portions thereof as antigens. For example, polyclonal and monoclonal antibodies can be produced by methods well known in the art, as described, for example, in Harlow and Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory (1988)), which is incorporated herein by reference. Invention polypeptides can be used as immunogens in generating such antibodies. Alternatively, synthetic peptides can be prepared (using commercially available synthesizers) and used as immunogens. Amino acid sequences can be analyzed by methods well known in the art to determine whether they encode hydrophobic or hydrophilic domains of the corresponding polypeptide. Altered antibodies such as chimeric, humanized, CDR-grafted or bifunctional antibodies can also be produced

by methods well known in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for example, in Sambrook et al., *supra.*, and Harlow and Lane, *supra*. Both anti-peptide and anti-fusion protein antibodies can be used. (see, for example, Bahouth et al., *Trends Pharmacol. Sci.* 12:338 (1991); Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley and Sons, NY (1989) which are incorporated herein by reference).

Invention antibodies also can be used to isolate invention polypeptides. Additionally the antibodies are useful for detecting the presence of invention polypeptides, as well as analysis of chromosome localization, and structural as well as functional domains. Methods for detecting the presence of SCA2 polypeptides on the surface of a cell comprise contacting the cell with an antibody that specifically binds to SCA2 polypeptides, under conditions permitting binding of the antibody to the polypeptides, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of invention polypeptides on the surface of the cell. With respect to the detection of such polypeptides, the antibodies can be used for *in vitro* diagnostic or *in vivo* imaging methods.

Immunological procedures useful for *in vitro* detection of target SCA2 polypeptides in a sample include immunoassays that employ a detectable antibody. Such immunoassays include, for example, ELISA, Pandex microfluorimetric assay, agglutination assays, flow cytometry, serum diagnostic assays and immunohistochemical staining procedures which are well known in the art. An antibody can be made detectable by various means well known in the art. For example, a detectable marker can be directly or indirectly attached

to the antibody. Useful markers include, for example, radionucleotides, enzymes, fluorogens, chromogens and chemiluminescent labels.

Further, invention antibodies can be used to modulate the activity of the SCA2 polypeptide in living animals, in humans, or in biological tissues or fluids isolated therefrom. Accordingly, compositions comprising a carrier and an amount of an antibody having specificity for SCA2 polypeptides effective to block binding of naturally occurring ligands to invention polypeptides. A monoclonal antibody directed to an epitope of SCA2 polypeptide molecules present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of an SCA2 polypeptide shown in SEQ ID NO:3, or SEQ ID NO:5, can be useful for this purpose.

The present invention further provides transgenic non-human mammals that are capable of expressing nucleic acids encoding SCA2 polypeptides. Also provided are transgenic non-human mammals capable of expressing nucleic acids encoding SCA2 polypeptides so mutated as to be incapable of normal activity, i.e., do not express native SCA2. The present invention also provides transgenic non-human mammals having a genome comprising antisense nucleic acids complementary to nucleic acids encoding SCA2 polypeptides so placed as to be transcribed into antisense mRNA complementary to mRNA encoding SCA2 polypeptides, which hybridizes thereto and, thereby, reduces the translation thereof. The nucleic acid may additionally comprise an inducible promoter and/or tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of nucleic acids are DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID NO:2, or SEQ ID NO:4. An

example of a non-human transgenic mammal is a transgenic mouse. Examples of tissue specificity-determining elements are the metallothionein promoter and the L7 promoter.

Animal model systems which elucidate the physiological and behavioral roles of SCA2 polypeptides are produced by creating transgenic animals in which the expression of the SCA2 polypeptide is altered using a variety of techniques. Examples of such techniques include the insertion of normal or mutant versions of nucleic acids encoding an SCA2 polypeptide by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos to produce a transgenic animal. (See, for example, Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Laboratory, (1986)).

Another technique, homologous recombination of mutant or normal versions of these genes with the native gene locus in transgenic animals, may be used to alter the regulation of expression or the structure of SCA2 polypeptides (see, Capecchi et al., *Science* 244:1288 (1989); Zimmer et al., *Nature* 338:150 (1989); which are incorporated herein by reference). Homologous recombination techniques are well known in the art. Homologous recombination replaces the native (endogenous) gene with a recombinant or mutated gene to produce an animal that cannot express native (endogenous) protein but can express, for example, a mutated protein which results in altered expression of SCA2 polypeptides.

In contrast to homologous recombination, microinjection adds genes to the host genome, without removing host genes. Microinjection can produce a

transgenic animal that is capable of expressing both endogenous and exogenous SCA2 protein. Inducible promoters can be linked to the coding region of nucleic acids to provide a means to regulate expression of the transgene. Tissue specific regulatory elements can be linked to the coding region to permit tissue-specific expression of the transgene. Transgenic animal model systems are useful for *in vivo* screening of compounds for identification of specific ligands, i.e., agonists and antagonists, which activate or inhibit protein responses.

Invention nucleic acids, oligonucleotides (including antisense), vectors containing same, transformed host cells, polypeptides and combinations thereof, as well as antibodies of the present invention, can be used to screen compounds *in vitro* to determine whether a compound functions as a potential agonist or antagonist to invention polypeptides. These *in vitro* screening assays provide information regarding the function and activity of invention polypeptides, which can lead to the identification and design of compounds that are capable of specific interaction with one or more types of polypeptides, peptides or proteins.

In accordance with still another embodiment of the present invention, there is provided a method for identifying compounds which bind to SCA2 polypeptides. The invention proteins may be employed in a competitive binding assay. Such an assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to SCA2 proteins. Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as modulators, agonists or antagonists of invention proteins.

In another embodiment of the invention, there is provided a bioassay for identifying compounds which modulate the activity of invention polypeptides. According to this method, invention polypeptides are contacted with an "unknown" or test substance (in the presence of a reporter gene construct when antagonist activity is tested), the activity of the polypeptide is monitored subsequent to the contact with the "unknown" or test substance, and those substances which cause the reporter gene construct to be expressed are identified as functional ligands for SCA2 polypeptides.

In accordance with another embodiment of the present invention, transformed host cells that recombinantly express invention polypeptides can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the SCA2-mediated response (via reporter gene expression) in the presence and absence of test compound, or by comparing the response of test cells or control cells (i.e., cells that do not express SCA2 polypeptides), to the presence of the compound.

As used herein, a compound or a signal that "modulates the activity" of invention polypeptides refers to a compound or a signal that alters the activity of SCA2 polypeptides so that the activity of the invention polypeptide is different in the presence of the compound or signal than in the absence of the compound or signal. In particular, such compounds or signals include agonists and antagonists. An agonist encompasses a compound or a signal that activates SCA2 protein expression. Alternatively, an antagonist includes a compound or signal that interferes with SCA2 protein expression. Typically, the effect of an antagonist is observed as a blocking of agonist-induced protein activation. Antagonists include competitive and non-competitive

antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for agonist binding. A non-competitive antagonist or blocker inactivates the function of the polypeptide by interacting with a site other than the agonist interaction site.

As understood by those of skill in the art, assay methods for identifying compounds that modulate SCA2 activity generally require comparison to a control. One type of a "control" is a cell or culture that is treated substantially the same as the test cell or test culture exposed to the compound, with the distinction that the "control" cell or culture is not exposed to the compound. For example, in methods that use voltage clamp electrophysiological procedures, the same cell can be tested in the presence or absence of compound, by merely changing the external solution bathing the cell. Another type of "control" cell or culture may be a cell or culture that is identical to the transfected cells, with the exception that the "control" cell or culture do not express native proteins. Accordingly, the response of the transfected cell to compound is compared to the response (or lack thereof) of the "control" cell or culture to the same compound under the same reaction conditions.

In yet another embodiment of the present invention, the activation of SCA2 polypeptides can be modulated by contacting the polypeptides with an effective amount of at least one compound identified by the above-described bioassays.

In accordance with another embodiment of the present invention, there are provided methods for diagnosing spinocerebellar Ataxia Type 2, said method comprising:

detecting, in said subject, a genomic or transcribed mRNA sequence having an expanded CAG repeat at a location corresponding to between nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6).

The number of CAG repeats required to indicate spinocerebellar Ataxia Type 2 is substantially above normal, preferably at least about 10-15 CAG repeats above normal, with at least 13 CAG repeats above normal being especially preferred. A normal amount of CAG repeats in the SCA2 gene (SEQ ID NO:2) has been found to be about 22, while 23 CAG repeats is occasionally observed. Thus, in a preferred diagnostic method, at least about 35 CAG repeats are detected between nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6), with the detection of 37 CAG repeats being especially preferred.

Although expansion of trinucleotide repeats is now recognized as an important mutational mechanism in humans and SCA2 represents the 6th disease in which expansion of a CAG trinucleotide repeat causes disease, there are several features of the SCA2 repeat that appear to be unique. In the other five CAG expansion diseases, the CAG repeats on normal chromosomes are highly polymorphic. Multiple alleles are detected and repeat sizes on normal chromosomes range from a low of 7 repeats in DRPLA to 40 repeats in SCA3/MJD. Heterozygosity for these CAG repeats in the normal population are in the range of 0.80 and above. It has been suggested that the extended normal alleles represent founder alleles which are predisposed to expansion.

The SCA2 repeat is highly unusual, because only two alleles are observed in the normal population. A common allele with 22 repeats is found on 92% of chromosomes, a rare second allele in 8% of chromosomes.

Expansion of the SCA2 CAG repeat on disease chromosomes is relatively moderate and is in the range seen with expansions in the SBMA and Huntington's Disease (HD) genes. The lowest number of repeats causing SCA2 was 36 and the most common disease allele had 37 repeats. Disease alleles showing 36 repeats have now clearly been established for HD (Rubinsztein et al., 1996, Am. J. Hum. Genet., 59:16-22), although normal elderly individuals with 36-40 repeats exist and the most common HD alleles have >40 repeats. In contrast to SCA1, where normal and disease alleles may differ by only one repeat unit, the longest normal and the shortest SCA2 disease allele are separated by 13 repeats. Once expanded on disease chromosomes, the SCA2 repeat may undergo moderate expansions.

The SCA2 repeat is contained in a novel gene which is transcribed in several tissues including non-neuronal tissues. The gene product, ataxin-2, has a predicted molecular weight of 140 kDa which is in good agreement with the 150 kDa protein observed using a monoclonal antibody to long polyglutamine tracts. A similar pattern of nearly ubiquitous expression has been observed in the other five polyglutamine diseases. Despite the phenotypic overlap of SCA2 with SCA1 and SCA3, the SCA2 gene shows no homology to these genes.

However, ataxin-2 showed significant homologies with another protein (referred to as "A2RP"; see Figure 7). A 42 amino acid domain was identified that was 86% identical between the two proteins. The potential functional importance of this domain was underscored by the fact that it was 100% conserved in the mouse SCA2 homologue (Figure 7). Interestingly, the polyglutamine tract was not conserved in either protein. Since the pathogenesis of polyglutamine containing proteins is still poorly understood, the identification of

functionally important domains adjacent to polyglutamine tracts may provide the potential for novel strategies to analyze the function of ataxin-2. A gain of function for the mutated ataxin-2 is supported by the fact that transcripts coding for mutated alleles are detected by RT-PCR.

Expansion of the SCA2 repeat appears to be a common cause of a dominant SCA phenotype in non-Portuguese patients. When samples from 45 families with SCA were screened, samples from 8 independent pedigrees showed expansion of the SCA2 repeat. It has been suggested that there are features specific to SCA2, but this assessment was limited to families large enough to be studied by linkage analysis. A better assessment of the range of SCA2 phenotypes is now possible due to the ability to test small families and single cases. In our patient sample, most patients had a `typical' SCA phenotype, but some patients had been classified as having an MJD phenotype and others showed a prominent dementia.

When performing direct testing for SCA2 mutations, great caution has to be exercised when interpreting the presence of expanded SCA2 alleles on polyacrylamide gels. A variable number of unrelated PCR fragments may be seen that are in the size range of expanded SCA2 repeats. Although these bands lack the typical `shadow' bands seen when di- or trinucleotide repeats are amplified, they may interfere with the interpretation in some samples. It is therefore recommended to confirm the presence of an expanded allele by Southern blotting and hybridization with a $(CAG)_{10}$ oligonucleotide.

In yet another embodiment of the present invention, there are provided methods for diagnosing spinocerebellar Ataxia Type 2, said method comprising:

a) contacting nucleic acid obtained from a subject suspected of having SCA2 with primers that amplify at least a nucleic acid fragment of SEQ ID NO:2 containing nucleotides 658-723 of SEQ ID NO:2, under conditions suitable to form a detectable amplification product; and

b) detecting an amplification product containing substantially expanded CAG repeats above normal, whereby said detection indicates that said subject has SCA2.

As indicated above, substantially expanded CAG repeats have at least about 10-15 CAG repeats above normal, with at least 13 CAG repeats above normal being especially preferred. Thus, in a preferred diagnostic method, at least about 35 CAG repeats are detected between nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6), with the detection of 37 CAG repeats being especially preferred.

In accordance with another embodiment of the present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid in a suitable packaging material. In one embodiment, the diagnostic nucleic acids are derived from SEQ ID NO:2 (Figure 6), preferably derived from nucleotides 163-657 and nucleotides 724-4098, with primers SCA2-A and SCA2-B being especially preferred. In another embodiment, the diagnostic nucleic acids are derived from SEQ ID NO:4. Invention diagnostic systems are useful for assaying for the presence or absence of the extended CAG repeat sequence between nucleotides 657 and 724 of SEQ ID NO:2 in the SCA2 gene in either genomic

DNA or in transcribed nucleic acid (such as mRNA or cDNA) encoding SCA2.

A suitable diagnostic system includes at least one invention nucleic acid, preferably two or more invention nucleic acids, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent are also typically included. Those of skill in the art can readily incorporate invention nucleic probes and/or primers into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein.

As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as invention nucleic acid probes or primers, and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention nucleic acids can be used for detecting a particular extended CAG repeat sequence between the region of genomic DNA corresponding to nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6), thereby diagnosing the presence of, or a predisposition for, spinocerebellar ataxia type 2. In addition, the packaging material contains instructions indicating how the materials within the kit are employed both to detect a particular sequence and diagnose the presence of, or a predisposition for, spinocerebellar ataxia type 2.

The packaging materials employed herein in relation to diagnostic systems are those customarily utilized in nucleic acid-based diagnostic systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the

like, capable of holding within fixed limits an isolated nucleic acid, oligonucleotide, or primer of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated nucleic acid, oligonucleotide or primer, or it can be a microtiter plate well to which microgram quantities of a contemplated nucleic acid probe have been operatively affixed.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

All U.S. patents and all publications mentioned herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting examples.

The invention will now be described in greater detail with reference to the following non-limiting examples.

Materials and Methods

Unless otherwise stated, the present invention was performed using standard procedures, as described, for example in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1982); Sambrook et al., *Molecular Cloning: A Laboratory Manual (2 ed.)*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1989); Davis et al., *Basic Methods in*

Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (1986); or *Methods in Enzymology: Guide to Molecular Cloning Techniques* Vol.152, S. L. Berger and A. R. Kimmerl Eds., Academic Press Inc., San Diego, USA (1987)).

Libraries. Yeast artificial chromosome (YAC) clones were obtained from the CEPH mega-YAC library and grown under standard conditions (Cohen et al., *Nature* 366:689-701 (1993)). *P1 artificial chromosome (PAC) library construction.* A 3X human PAC library, designated RPCI-1 (Ioannou et al., *Hum. Genet.* **219-220** (1994b)) was constructed as described (Ioannou et al., *Nat. Genet.* 6:84-89 (1994a)). The library was arrayed in 384 well dishes. Pools from portion of the library were screened by PCR with AFM154TC5 (D12S1333) and AFMa128yf1 (D12S1332). Subsequently, STSs generated by sequencing of clones using vector primers were used as hybridization probes to gridded colony filters of the PAC library.

YAC DNA preparation. YAC clones were grown in selective media, pelleted and resuspended in 3 ml 0.9 M sorbitol, 0.1M EDTA pH 7.5, then incubated with 100 U of lytocase (Sigma) at 37°C for 1 hour. After centrifugation for 5 minutes at 5,000 rpm pellets were resuspended in 3 ml 50 mM Tris pH 7.45, 20 mM EDTA three-tenth ml 10% SDS was added and the mixture was incubated at 65°C for 30 minutes. One ml of 5 M potassium acetate was added and tubes were left on ice for 1 hour, then centrifuged at 10,000 rpm for 10 minutes. Supernatant was precipitated in 2 volumes of ethanol and pelleted at 6,000 rpm for 15 minutes. Pellets were resuspended in TE, treated with RNase and reextracted with phenol-chloroform.

Analysis by pulsed-field gel electrophoresis. Agarose plugs of yeast cells containing total YAC DNA were prepared (Larin and Lehrach, *Genet. Rcs.* 56:203-208 (1990)) and subjected to pulsed-field gel separation on 1% SeaKem agarose gels in 0.5X TBE using the CHEF DRII Mapper (Bio-Rad). PAC and BAC clones were sized after digestion with XbaI and NotI. Gels were blotted onto Magna NT Nylon membranes using alkaline blotting, UV cross linked and baked at 80°C for two hours. Membranes were hybridized with total human DNA, washed according to standard procedures, and exposed to Kodak XAR5 film. The sizes of individual clones were determined by comparison to their relative positions with molecular weight standards.

Analysis by fluorescence in situ hybridization (FISH). PAC or BAC clones were biotinylated by nicktranslation in the presence of biotin-14-dATP using the BioNick Labeling Kit (Gibco-BRL). FISH was performed essentially as described (Korenberg et al., *Cytogenet Cell Genet.* 69:196-200 (1995)). Briefly, 400 ng of probe DNA was mixed with 8 ng of human Cot 1 DNA (Gibco-BRL) and 2 ug of sonicated salmon sperm DNA in order to suppress possible background produced from repetitive human sequences as well as yeast sequences in the probe. The probes were denatured at 75°C, preannealed at 37°C for one hour, and applied to denatured chromosome slides prepared from normal male lymphocytes (Korenberg et al., 1995, supra). Post-hybridization washes were performed at 40°C in 2X SSC/50% formamide followed by washes in 1X SSC at 50°C. Hybridized DNAs were detected with avidin-conjugated fluorescent isothiocyanate (Vector Laboratories). One amplification was performed by using biotinylated anti-avidin. For distinguishing chromosome subbands precisely, a reverse banding technique was used, which was achieved by chromomycin A3 and distamycin A

double staining (Korenberg et al., 1995, supra). The color images were captured by using a Photometrics Cooled-CCD camera and BDS image analysis software (Oncor Imaging, Inc.).

PAC and BAC DNA preparation. Selected clones were grown overnight in LB media containing 12.5 μg/ml kanamycin for PACs and 12.5 μg/ml chloramphenicol for BACs. DNAs were prepared by the alkaline lysis method. PAC DNAs were digested with *Not*I and subjected to pulsed-field gel electrophoresis. Sizes were determined relative to λ concatamers.

Southern blot analysis. Gel electrophoresis of DNA was carried out on 0.8% agarose gels in 1x TBE. Transfer of nucleic acids to Nybond N+ nylon membrane (Amersham) was performed according to the manufacturer's instruction. Probes were labelled using RadPrime Labeling System (BRL). Hybridization was carried out at 42°C for 16 hours in 50% formamide, 5x SSPE, 5x Denhardt's 0.1% SDS, 100 mg/ml denatured salmon sperm DNA. The filters were washed once in 1x SSC, 0.1% SDS at room temperature for 20 minutes, and twice in 0.1x SSC, 0.1% SDS for 20 minutes at 65°C. The blots were exposed onto X-ray film (Kodak, X-OMAT-AR).

Sequencing of PAC endclones. PAC clones were inoculated into 500 ml of LB/kanamycin and grown overnight. DNAs were isolated using QIAGEN columns according to the vendors protocol with one additional phenol/chloroform/isoamylalcohol extraction followed by one additional chloroform/isoamylalcohol extraction. Clones were sequenced using the Gibco-BRL cycle sequencing kit with standard T7 and SP6 primers.

Hybridization of $(CAG)_{10}$ oligonucleotides. Eighty ng of oligonucleotide were 5' end-labeled and hybridized overnight at 42°C in buffer containing 1 M NaCl, 0.05 M Tris HCl pH7, 5.5 mM EDTA, 0.1 % SDS, 1X Denhardt's solution and 200 µg/ml denatured salmon sperm DNA. Filters were washed 2 times with 2X SSC, 0.1% SDS at 55°C and exposed to Kodak X-ray film for 24 hours, and subsequently washed at 65°C, followed by additional exposure to X-ray film.

Regression Analysis. The data were fit using the Statistical Analysis Software (SAS) package version 3.10 using the Secant Method (Ralston et al, 1978, Technometrics, 20:7-14). The regression equation was $y=A*\exp(-ax)$, where y gives the age of onset and x the number of CAG repeats. The conversion criteria were met with the mean square error of 76.598. The value of parameters are as follows: A=1171.583, a=0.091.

EXAMPLE 1

Physical Map of the SCA2 region

BAC library construction of total human genomic DNA was performed as described in Shizuya et al., *Proc. Natl. Acad. Sci. USA* 89:8794-8797 (1992). BAC clones were screened by PCR using STSs (D12S1228, S29, S32, S33). Insert size of clones was measured by running pulsed-field gel electrophoresis after digesting DNA with NotI.

The marker AFMa128yf1 (D12S1332) which was non-recombinant in several SCA2 pedigrees served as the starting point to assemble a PAC contig. This was done by screening PCR pools of a 3x human PAC library (Ioannou et al., 1994). Two clones were positive for this STS (Fig. 1). Single copy sequences from PAC ends were obtained from P168L1 and used to extend this contig.

Subsequent 'walking steps, however, were undertaken by hybridizing PCR-generated STS fragments to gridded membranes of the 3x PAC library and the 1x total human genome BAC library (Research Genetics).

In a similar fashion, a second contig was established starting with the telomeric flanking marker AFM154tc5 (D12S1333). A total of two clones were identified by screening of PCR pools. After several walking steps, overlap of the two contigs was established by shared STSs (Fig. 1) and by shared restriction fragments (data not shown). All STSs shown in Fig. 1 were mapped back to human chromosome 12 by PCR analysis of a human/Chinese hamster somatic hybrid cell line, HHW582, which contains CHR 12 as the only human chromosome, and by analysis of a chromosome 12 specific lambda library, LL12NS01 (both from Coriell Cell Repositories). Map position in 21q24.1 for clones B295CO5, P191C5 and P65I22 was confirmed using FISH (Fig. 1b).

At the same time contigs were constructed for the other flanking markers AFM240wel (D12S1328), AFM291xe9 (D12S1329), and markers WI-4176 and WI-6850 (data not shown). These contigs did not overlap with one another, nor with the AFMal28yf1/AFM154tc5 contig.

All PAC and BAC clones were sized by pulsed-field electrophoresis after digestion with NotI. Overlap of clones was initially determined by shared STS content, and subsequently confirmed by hybridization of selected clones to Southern blots of NotI/XbaI digests of clones.

The dense localization of STSs allowed the precise positioning of YACs that had been identified by screening of PCR pools of the CEPH mega-YAC library with either AFMa128yf1 or AFM154tc5. The only YAC that was

positive for both AFMa128yf1 (D12S1332) and AFM154tc5, Y884_h_11, contained an approximately 200 kb interstitial deletion. A small portion of this deletion was not covered by any of the other YAC clones.

EXAMPLE 2

Identification of SCA2-related trinucleotide repeats

Since we had observed marked anticipation in one pedigree with SCA2, we identified clones containing trinucleotide repeats. EcoRI digests of a minimal tiling path of PAC clones were hybridized with a $(CAG)_{10}$ nucleotide, as well as other trinucleotide permutations. Three CAG positive bands of distinct sizes were identified in the contig.

PAC clone P65I22 was digested with Sau3A and subcloned into the pBluescript SK (+) phagemid (Stratagene). After transfection into DH5α, bacterial colonies were screened for poly-CAG containing inserts using the methods described above. Positive clones were sequenced using the Circum Vent cycle sequencing kit (New England Biolabs) with end-labeled T3 and T7 primers. However, no reliable sequence could be obtained from the initial plasmid PL65I22. Therefore, this plasmid was digested with BssHII, recloned into the pBluescript plasmid, and CAG-positive clones sequenced with primers corresponding to the following nucleotides of the vector sequence (primer A: 828-848, primer B: 547-565). The sequence of this plasmid, designated PL65I22B, allowed the generation of primers SCA2-A and SCA2-B, which were used to confirm the sequence flanking the CAG repeat.

Plasmid PL65I22B containing an extended CAG repeat that appeared to be embedded into a long open reading frame (ORF) (Figure 2; SEQ ID NO:1). Sequence analysis of this plasmid appeared to be extremely

difficult due to the abundant presence of premature terminations (see below). The CAG repeat in PL65I22B was twice interrupted and had the following structure $(CAG)_8CAA(CAG)_4CAA(CAG)_8$. Four additional PAC clones and one BAC clone contained the SCA2 repeat, and all clones had 22 repeats with two CAA interruptions. Analysis of the genomic DNA sequence flanking the CAG repeat suggested the presence of an open reading frame (see also Figure 6) and a potential splice site 3' of the CAG repeat (vertical arrow in Figure 2).

The difficulties encountered in sequencing this region suggested that stable secondary structures might be formed in this GC-rich region. Previous analysis of trinucleotide repeats predisposed to expansion had suggested that these regions are predicted to form hairpin structures. We used an up-dated version of the DNA-FOLD Program (SantaLucia et al., 1996, Biochemistry , 35:3555-3562) for secondary structure predictions.

Subsequent analysis of the sequence flanking the CAG repeat using the OLIGO Program indicated that it contained several palindromic sequences predicted to form hairpin lcops. Despite the predicted hairpin structures sufficient sequence information was generated to design primers flanking the CAG repeat for the PCR analysis of patient samples.

Example 3

Genomic analysis of an extended CAG SCA2 repeat

Using primer pairs SCA2-A and B, genomic DNAs from normal controls and SCA2 patients were amplified and separated by agarose gel electrophoresis. The best results were obtained at an annealing temperature of 63°C with denaturation times of 90 sec.

Eighty ng each of primers SCA2-A (5'-GGG CCC CTC ACC ATG TCG-3') and SCA2-B (5'-CGG GCT TGC GGA CAT TGG-3') were added to 20 ng of human DNA with standard PCR buffer and nucleotide concentrations. After an initial denaturation at 95°C for 5 minutes, 35 cycles were repeated with denaturation at 96°C for 1.5 minutes, an annealing temperature of 63°C for 30 seconds, extension at 72°C for 1.5 minutes, and a final extension of 5 minutes at 72°C.

PCR products obtained by PCR amplification of genomic DNAs were separated by electrophoresis through 2% agarose gels in 1x TBE buffer at 10 V/cm. Gels were transferred to nylon membranes (MSI, Westborough, MA) using standard procedures for Southern blotting. Membranes were hybridized with a $(CAG)_{10}$ oligonucleotide and processed as described above.

On agarose electrophoresis, a single band of approximately 130 bp was detected in 20 normal individuals, although occasionally two closely spaced bands could be observed. In contrast, all 15 patients with SCA2 from 3 independent famalies showed one allele in the normal size range and a larger allele ranging from approximately 190 to 250 bp. Southern blot analysis confirmed that both alleles contained CAG repeats.

To determine the exact sizes of amplified fragments, DNAs from SCA2 patients and 50 normal individuals were amplified and PCR products separated by polyacrylamide gel electrophoresis. A common allele of 22 repeats and a less frequent allele of 23 repeats were observed on normal chromosomes (Figure 3). The allele frequencies were 0.92 for the smaller and 0.08 for the larger allele. In patients from three independent SCA2 pedigrees, however, extended alleles ranging from 36 to 52 repeats were observed (Figure 3). Once expanded to

the pathologic range, the SCA2 repeat was moderately unstable and further expansion by 2 to 9 repeat units was observed during meiosis (Figure 3). There was great variability of the age of onset for a given repeat length, especially for disease alleles with 36-40 repeats (Figure 4). Due to the heterogeneous variance of age of onset we used non-linear regression, and an exponential function was successfully fitted (see methods and Figure 4). The smallest expansion of 36 repeats was seen in two men with disease onset at ages 37 and 44. The longest expansion of 52 repeats was seen in a boy with disease onset at 9 years of age.

Sequence analysis of ten normal alleles revealed that the common normal allele with 22 repeats contained the two CAA interruptions that were also detected in plasmid PL65I22B. The less frequent normal allele with 23 repeats had lost the 5' CAA interruption, and contained an additional CAG repeat at the 5'-end of the repeat. In three expanded alleles that were isolated from SCA2 patients the CAG repeat lacked any interruptions.

To determine the frequency of mutation in the SCA2 gene in non-Portuguese patients we screened DNAs from 45 independent families with autosomal dominant SCAs. Expansion of the SCA2 repeat was detected in six families. In this set of families, SCA2 expansion was twice as common as expansion in the SCA1 gene. In addition to individuals with a `typical' SCA phenotype, expansion of the SCA2 repeat was detected in a pedigree with a MJD phenotype and one family with SCA and marked dementia.

EXAMPLE 4

Isolation of human SCA2 cDNA

cDNA library screen: ^{32}P-labeled probes were generated by PCR amplification of plasmid P65I22B using the following primer pair: 65A3: 5'CCGCGGCTGCCAATGTCC, 65B5: 5'GTAACCGTTCGGCCCCG. A second probe was generated using primers 65A6: 5'GGCTCCCGGCGGCTCCTT; 65B6: 5'TGCTGCTGCTGCTGGGGCTTCAG. Screening of the trisomy 21 fetal brain cDNA library and the Stratagene adult human frontal cortex cDNA Lamba Zap II library was performed using the amplification products generated from plasmid P65I22B. Phages were plated to an average density of 1 x 10^5 per 150 cm^2 plate. Plaque lifts of 20 plates (2 x 10^6 phages) were made using duplicated nylon membranes (Duralose-UV, Stratagene). Hybridization and excision were performed according to the manufacturer's protocol. Hybridized membranes were washed to a final stringency of 0.2x SSC, 0.1x SDS at 65C. The filters were exposed overnight onto X-ray film. Excised phagemids were grown overnight in 5ml LB medium containing 50 ug/ml of ampicillin.

Using PCR-generated fragments containing nucleotides 39-237 and 262 to 397 (according to the sequence shown in Figure 2) we initially screened a human adult frontal cortex library (Stratagene). Through screening of 0.8 x 10^6 clones, two positive clones, S1 and S2, were identified. To obtain additional clones, 2x10^6 clones of a human fetal brain library generated from a fetus with trisomy 21 (Yamakawa et al., 1995, Hum. Mol. Genet., 4:709-716) were screened using the same PCR-generated fragments. A total of 15 clones were obtained, all of which were partially sequenced to determine alignment of clones. These clones appeared to belong to a total of two classes of clones (designated F1.1 through F1.7 and F2.1 through F2.8) that contained long portions

of the 3' untranslated region and a poly-A tail (Figure 5). Both classes of clones extended 40 and 265 bp 5' of the CAG repeat in the coding region of the SCA2 gene.

To obtain cDNA sequence for the 5' end of the SCA2 coding region, placental poly-T selected placental mRNAs (Clontech) were transcribed with MMLV reverse transcriptase and amplified with the following primer pairs: SCA2-A30: 5'CCGCCCGCTCCTCACGTGT, SCA2-A31: 5'ACCCCCGAGAAAGCAACC; SCA2-B30: 5'-CCGTTGCCGTTGCTACCA. The sequences for primers SCA2-A30 and A31 were obtained from genomic sequence, and are located 5' to the stop codon preceding the putative initiator methionine. The sequence for SCA2-B30 was obtained from the 5' end of cDNA clones F1.1 and F1.2. The amplicons obtained by RT-PCR were directly sequenced.

The composite of the human SCA2 cDNA sequence assembled from several overlapping cDNA clones is shown in Figure 6 (SEQ ID NO:2). The longest open reading frame consists of 3936 bp and ends with a TAA termination codon. The stop codon is followed by 364 bp of 3' untranslated sequence. The CAG repeat is located in the 5'end of the coding region. The putative translation start site follows an in frame stop codon located 78 bp upstream. The predicted molecular weight for the SCA2 translation product is 140.1 kDa with the CAG trinucleotide repeat predicted to code for glutamine. In analogy to the SCA1 gene product, we propose the name ataxin-2 for the SCA2 gene product.

The cDNA sequence was compared against the GenBank database using the FASTA sequence alignment algorithms and the TIGR database. The predicted protein sequence was compared against the SwissProt database and the predicted translation products of the GenBank database. These searches revealed no significant

similarities to genes of known function except for limited homologies to the GLI-Krueppel related protein YY1 (nucleotides 45 to 586, odds against chance occurrence 6.6×10^{-7}).

However, significant similarities were detected with two partial cDNA transcripts in the TIGR database (THC148678, H03566, odds against chance similarity $<10^{-31}$). Complete sequence analysis of these cDNA clones (purchased from ATCC) revealed significant homologies with ataxin-2. This protein was named ataxin-2 related protein (A2RP). The region showing the most significant homology including a domain of 42 amino acids with 86% identity (codons 243-284 of the consensus sequence) is shown in Figure 7. This domain is also 100% conserved in mouse ataxin-2. Despite the significant homologies, the polyglutamine tract in ataxin-2 was replaced with an interrupted polyproline tract in the related A2RP human protein and was reduced to one glutamine in the mouse SCA2 homologue (see Figure 7).

Example 6

RT-PCR and Northern blot analysis:

RNA isolation and reverse transcription was carried out using well-known methods (Huynh et al., 1994, Hum. Mol. Genet.,3:1075-1079). RNAs were isolated from lymphoblastoid cell lines established from patients and unrelated spouses in the FS pedigree with SCA2 (Pulst et al., 1993, Nat. Genet., 5:8-10). Multiple tissue Northern blots were purchased from Clontech. For amplification, primers located in two exons (SCA-A and SCA-B14, see also Figure 6) were chosen so that genomic DNA was not amplified. The sequence for SCA-B14 was: 5'TTCTCATGTGCGGCATCAAG.

Using RT-PCR, it was determined that the SCA2 CAG repeat was transcribed in lymphoblastoid cell lines. In cDNAs from SCA2 patients, transcription from both the normal and the expanded allele was detected using oligonucleotide primers that flank the repeat. By Northern blot analysis, the SCA2 gene was determined to be widely expressed. A strong signal corresponding to a 4.5 kb transcript was detected in all brain regions examined. This transcript was also detected in RNAs isolated from heart, placenta, liver, skeletal muscle, and pancreas. Little transcript was detected in lung and no transcription was detectable in kidney. A much fainter transcript of 7.5 kb could be seen in RNAs isolated from some brain regions and in some peripheral tissues.

EXAMPLE 7

Isolation of mouse SCA2 cDNA

To identify mouse SCA2 cDNA clones, the Stratagene Lambda ZAP newborn mouse brain cDNA library was screened with a human SCA2 cDNA clone. Six clones were identified and sequenced. A full-length mouse SCA2 cDNA is set forth in SEQ ID NO:4.

SUMMARY OF SEQUENCES

SEQ ID NO:1 is the genomic nucleic acid sequence set forth in Figure 2.

SEQ ID NO:2 is the nucleic acid sequence (and the deduced amino acid sequence) of a cDNA encoding a human-derived SCA2 protein of the present invention (also set forth in Figure 6).

SEQ ID NO:3 is the deduced amino acid sequence of the human-derived SCA2 protein set forth in SEQ ID NO:2.

SEQ ID NO:4 is the nucleic acid sequence (and the deduced amino acid sequence) of a cDNA encoding a mouse-derived SCA2 protein of the present invention.

SEQ ID NO:5 is the deduced amino acid sequence of the mouse-derived SCA2 protein set forth in SEQ ID NO:4.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CEDARS-SINAI MEDICAL CENTER

(ii) TITLE OF INVENTION: NUCLEIC ACID ENCODING SPINOCEREBELLAR ATAXIA-2 AND PRODUCTS RELATED THERETO

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Campbell & Flores LLP
(B) STREET: 4370 La Jolla Village Drive, Suite 700
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Ramos, Robert T.
(B) REGISTRATION NUMBER: 37,915
(C) REFERENCE/DOCKET NUMBER: FP CE 2563

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 535-9001
(B) TELEFAX: (619) 535-8949

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 516 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGGTAGCAA CGGAAACGGC GGCGGCGCGT TTCGGCCCGG CTCCCGGCGG CTCCTTGGTC 60

TCGGCGGGCC TCCCCGCCCC TTCGTCGTCG TCCTTCTCCC CCTCGCCAGC CCGGGCGCCC 120

CTCCGGCCGC GCCAACCCGC GCCTCCCCGC TCGGCGCCCG TGCGTCCCCG CCGCGTTCCG 180

GCGTCTCCTT GGCGCGCCCG GCTCCCGGCT GTCCCCGCCC GGCGTGCGAG CCGGTGTATG 240

GGCCCCTCAC CATGTCGCTG AAGCCCCAGC AGCAGCAGCA GCAGCAGCAG CAACAGCAGC 300

AGCAGCAACA GCAGCAGCAG CAGCAGCAGC AGCCGCCGCC CGCGGCTGCC AATGTCCGCA 360

AGCCCGGCGG CAGCGGCCTT CTAGCGTCGC CCGCCGCCGC GCCTTCGCCG TCCTCGTCCT 420

CGGTCTCCTC GTCCTCGGCC ACGGCTCCCT CCTCGGTGGT CGCGGCGACC TCCGGCGGCG 480

GGAGGCCCGG CCTGGGCAGG TGGGTGTCGG CACCCC 516

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4481 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 163..4101

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACCCCCGAGA AAGCAACCCA GCGCGCCGCC CGCTCCTCAC GTGTCCCTCC CGGCCCCGGG 60

GCCACCTCAC GTTCTGCTTC CGTCTGACCC CTCCGACTTC CGGTAAAGAG TCCCTATCCG 120

CACCTCCGCT CCCACCCGGC GCCTCGGCGC GCCCGCCCTC CG ATG CGC TCA GCG 174
Met Arg Ser Ala
1

GCC GCA GCT CCT CGG AGT CCC GCG GTG GCC ACC GAG TCT CGC CGC TTC 222
Ala Ala Ala Pro Arg Ser Pro Ala Val Ala Thr Glu Ser Arg Arg Phe
5 10 15 20

GCC GCA GCC AGG TGG CCC GGG TGG CGC TCG CTC CAG CGG CCG GCG CGG 270
Ala Ala Ala Arg Trp Pro Gly Trp Arg Ser Leu Gln Arg Pro Ala Arg
25 30 35

CGG AGC GGG CGG GGC GGC GGT GGC GCG GCC CCG GGA CCG TAT CCC TCC 318
Arg Ser Gly Arg Gly Gly Gly Gly Ala Ala Pro Gly Pro Tyr Pro Ser
40 45 50

GCC GCC CCT CCC CCG CCC GGC CCC GGC CCC CCT CCC TCC CGG CAG AGC 366
Ala Ala Pro Pro Pro Pro Gly Pro Gly Pro Pro Pro Ser Arg Gln Ser
55 60 65

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TCG CCT CCC TCC GCC TCA GAC TGT TTT GGT AGC AAC GGC AAC GGC GGC      414
Ser Pro Pro Ser Ala Ser Asp Cys Phe Gly Ser Asn Gly Asn Gly Gly
 70                  75                  80

GGC GCG TTT CGG CCC GGC TCC CGG CGG CTC CTT GGT CTC GGC GGG CCT      462
Gly Ala Phe Arg Pro Gly Ser Arg Arg Leu Leu Gly Leu Gly Gly Pro
 85                  90                  95                 100

CCC CGC CCC TTC GTC GTC GTC CTT CTC CCC CTC GCC AGC CCG GGC GCC      510
Pro Arg Pro Phe Val Val Val Leu Leu Pro Leu Ala Ser Pro Gly Ala
                105                 110                 115

CCT CCG GCC GCG CCA ACC CGC GCC TCC CCG CTC GGC GCC CGT GCG TCC      558
Pro Pro Ala Ala Pro Thr Arg Ala Ser Pro Leu Gly Ala Arg Ala Ser
            120                 125                 130

CCG CCG CGT TCC GGC GTC TCC TTG GCG CGC CCG GCT CCC GGC TGT CCC      606
Pro Pro Arg Ser Gly Val Ser Leu Ala Arg Pro Ala Pro Gly Cys Pro
        135                 140                 145

CGC CCG GCG TGC GAG CCG GTG TAT GGG CCC CTC ACC ATG TCG CTG AAG      654
Arg Pro Ala Cys Glu Pro Val Tyr Gly Pro Leu Thr Met Ser Leu Lys
    150                 155                 160

CCC CAG CAG CAG CAG CAG CAG CAG CAG CAA CAG CAG CAG CAG CAA CAG      702
Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
165                 170                 175                 180

CAG CAG CAG CAG CAG CAG CAG CCG CCG CCC GCG GCT GCC AAT GTC CGC      750
Gln Gln Gln Gln Gln Gln Gln Pro Pro Pro Ala Ala Ala Asn Val Arg
                185                 190                 195

AAG CCC GGC GGC AGC GGC CTT CTA GCG TCG CCC GCC GCC GCG CCT TCG      798
Lys Pro Gly Gly Ser Gly Leu Leu Ala Ser Pro Ala Ala Ala Pro Ser
            200                 205                 210

CCG TCC TCG TCC TCG GTC TCC TCG TCC TCG GCC ACG GCT CCC TCC TCG      846
Pro Ser Ser Ser Ser Val Ser Ser Ser Ser Ala Thr Ala Pro Ser Ser
        215                 220                 225

GTG GTC GCG GCG ACC TCC GGC GGC GGG AGG CCC GGC CTG GGC AGA GGT      894
Val Val Ala Ala Thr Ser Gly Gly Gly Arg Pro Gly Leu Gly Arg Gly
    230                 235                 240

CGA AAC AGT AAC AAA GGA CTG CCT CAG TCT ACG ATT TCT TTT GAT GGA      942
Arg Asn Ser Asn Lys Gly Leu Pro Gln Ser Thr Ile Ser Phe Asp Gly
245                 250                 255                 260

ATC TAT GCA AAT ATG AGG ATG GTT CAT ATA CTT ACA TCA GTT GTT GGC      990
Ile Tyr Ala Asn Met Arg Met Val His Ile Leu Thr Ser Val Val Gly
                265                 270                 275

TCC AAA TGT GAA GTA CAA GTG AAA AAT GGA GGT ATA TAT GAA GGA GTT     1038
Ser Lys Cys Glu Val Gln Val Lys Asn Gly Gly Ile Tyr Glu Gly Val
            280                 285                 290
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TTT AAA ACT TAC AGT CCG AAG TGT GAT TTG GTA CTT GAT GCC GCA CAT      1086
Phe Lys Thr Tyr Ser Pro Lys Cys Asp Leu Val Leu Asp Ala Ala His
        295                 300                 305

GAG AAA AGT ACA GAA TCC AGT TCG GGG CCG AAA CGT GAA GAA ATA ATG      1134
Glu Lys Ser Thr Glu Ser Ser Ser Gly Pro Lys Arg Glu Glu Ile Met
    310                 315                 320

GAG AGT ATT TTG TTC AAA TGT TCA GAC TTT GTT GTG GTA CAG TTT AAA      1182
Glu Ser Ile Leu Phe Lys Cys Ser Asp Phe Val Val Val Gln Phe Lys
325                 330                 335                 340

GAT ATG GAC TCC AGT TAT GCA AAA AGA GAT GCT TTT ACT GAC TCT GCT      1230
Asp Met Asp Ser Ser Tyr Ala Lys Arg Asp Ala Phe Thr Asp Ser Ala
                345                 350                 355

ATC AGT GCT AAA GTG AAT GGC GAA CAC AAA GAG AAG GAC CTG GAG CCC      1278
Ile Ser Ala Lys Val Asn Gly Glu His Lys Glu Lys Asp Leu Glu Pro
            360                 365                 370

TGG GAT GCA GGT GAA CTC ACA GCC AAT GAG GAA CTT GAG GCT TTG GAA      1326
Trp Asp Ala Gly Glu Leu Thr Ala Asn Glu Glu Leu Glu Ala Leu Glu
        375                 380                 385

AAT GAC GTA TCT AAT GGA TGG GAT CCC AAT GAT ATG TTT CGA TAT AAT      1374
Asn Asp Val Ser Asn Gly Trp Asp Pro Asn Asp Met Phe Arg Tyr Asn
    390                 395                 400

GAA GAA AAT TAT GGT GTA GTG TCT ACG TAT GAT AGC AGT TTA TCT TCG      1422
Glu Glu Asn Tyr Gly Val Val Ser Thr Tyr Asp Ser Ser Leu Ser Ser
405                 410                 415                 420

TAT ACA GTG CCC TTA GAA AGA GAT AAC TCA GAA GAA TTT TTA AAA CGG      1470
Tyr Thr Val Pro Leu Glu Arg Asp Asn Ser Glu Glu Phe Leu Lys Arg
                425                 430                 435

GAA GCA AGG GCA AAC CAG TTA GCA GAA GAA ATT GAG TCA AGT GCC CAG      1518
Glu Ala Arg Ala Asn Gln Leu Ala Glu Glu Ile Glu Ser Ser Ala Gln
            440                 445                 450

TAC AAA GCT CGA GTG GCC CTG GAA AAT GAT GAT AGG AGT GAG GAA GAA      1566
Tyr Lys Ala Arg Val Ala Leu Glu Asn Asp Asp Arg Ser Glu Glu Glu
        455                 460                 465

AAA TAC ACA GCA GTT CAG AGA AAT TCC AGT GAA CGT GAG GGG CAC AGC      1614
Lys Tyr Thr Ala Val Gln Arg Asn Ser Ser Glu Arg Glu Gly His Ser
    470                 475                 480

ATA AAC ACT AGG GAA AAT AAA TAT ATT CCT CCT GGA CAA AGA AAT AGA      1662
Ile Asn Thr Arg Glu Asn Lys Tyr Ile Pro Pro Gly Gln Arg Asn Arg
485                 490                 495                 500

GAA GTC ATA TCC TGG GGA AGT GGG AGA CAG AAT TCA CCG CGT ATG GGC      1710
Glu Val Ile Ser Trp Gly Ser Gly Arg Gln Asn Ser Pro Arg Met Gly
                505                 510                 515
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CAG CCT GGA TCG GGC TCC ATG CCA TCA AGA TCC ACT TCT CAC ACT TCA      1758
Gln Pro Gly Ser Gly Ser Met Pro Ser Arg Ser Thr Ser His Thr Ser
            520                 525                 530

GAT TTC AAC CCG AAT TCT GGT TCA GAC CAA AGA GTA GTT AAT GGA GGT      1806
Asp Phe Asn Pro Asn Ser Gly Ser Asp Gln Arg Val Val Asn Gly Gly
        535                 540                 545

GTT CCC TGG CCA TCG CCT TGC CCA TCT CCT TCC TCT CGC CCA CCT TCT      1854
Val Pro Trp Pro Ser Pro Cys Pro Ser Pro Ser Ser Arg Pro Pro Ser
    550                 555                 560

CGC TAC CAG TCA GGT CCC AAC TCT CTT CCA CCT CGG GCA GCC ACC CCT      1902
Arg Tyr Gln Ser Gly Pro Asn Ser Leu Pro Pro Arg Ala Ala Thr Pro
565                 570                 575                 580

ACA CGG CCG CCC TCC AGG CCC CCC TCG CGG CCA TCC AGA CCC CCG TCT      1950
Thr Arg Pro Pro Ser Arg Pro Pro Ser Arg Pro Ser Arg Pro Pro Ser
                585                 590                 595

CAC CCC TCT GCT CAT GGT TCT CCA GCT CCT GTC TCT ACT ATG CCT AAA      1998
His Pro Ser Ala His Gly Ser Pro Ala Pro Val Ser Thr Met Pro Lys
            600                 605                 610

CGC ATG TCT TCA GAA GGG CCT CCA AGG ATG TCC CCA AAG GCC CAG CGA      2046
Arg Met Ser Ser Glu Gly Pro Pro Arg Met Ser Pro Lys Ala Gln Arg
        615                 620                 625

CAT CCT CGA AAT CAC AGA GTT TCT GCT GGG AGG GGT TCC ATA TCC AGT      2094
His Pro Arg Asn His Arg Val Ser Ala Gly Arg Gly Ser Ile Ser Ser
    630                 635                 640

GGC CTA GAA TTT GTA TCC CAC AAC CCA CCC AGT GAA GCA GCT ACT CCT      2142
Gly Leu Glu Phe Val Ser His Asn Pro Pro Ser Glu Ala Ala Thr Pro
645                 650                 655                 660

CCA GTA GCA AGG ACC AGT CCC TCG GGG GGA ACG TGG TCA TCA GTG GTC      2190
Pro Val Ala Arg Thr Ser Pro Ser Gly Gly Thr Trp Ser Ser Val Val
                665                 670                 675

AGT GGG GTT CCA AGA TTA TCC CCT AAA ACT CAT AGA CCC AGG TCT CCC      2238
Ser Gly Val Pro Arg Leu Ser Pro Lys Thr His Arg Pro Arg Ser Pro
            680                 685                 690

AGA CAG AAC AGT ATT GGA AAT ACC CCC AGT GGG CCA GTT CTT GCT TCT      2286
Arg Gln Asn Ser Ile Gly Asn Thr Pro Ser Gly Pro Val Leu Ala Ser
        695                 700                 705

CCC CAA GCT GGT ATT ATT CCA ACT GAA GCT GTT GCC ATG CCT ATT CCA      2334
Pro Gln Ala Gly Ile Ile Pro Thr Glu Ala Val Ala Met Pro Ile Pro
    710                 715                 720

GCT GCA TCT CCT ACG CCT GCT AGT CCT GCA TCG AAC AGA GCT GTT ACC      2382
Ala Ala Ser Pro Thr Pro Ala Ser Pro Ala Ser Asn Arg Ala Val Thr
725                 730                 735                 740
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CCT TCT AGT GAG GCT AAA GAT TCC AGG CTT CAA GAT CAG AGG CAG AAC      2430
Pro Ser Ser Glu Ala Lys Asp Ser Arg Leu Gln Asp Gln Arg Gln Asn
                745                 750                 755

TCT CCT GCA GGG AAT AAA GAA AAT ATT AAA CCC AAT GAA ACA TCA CCT      2478
Ser Pro Ala Gly Asn Lys Glu Asn Ile Lys Pro Asn Glu Thr Ser Pro
            760                 765                 770

AGC TTC TCA AAA GCT GAA AAC AAA GGT ATA TCA CCA GTT GTT TCT GAA      2526
Ser Phe Ser Lys Ala Glu Asn Lys Gly Ile Ser Pro Val Val Ser Glu
        775                 780                 785

CAT AGA AAA CAG ATT GAT GAT TTA AAG AAA TTT AAG AAT GAT TTT AGG      2574
His Arg Lys Gln Ile Asp Asp Leu Lys Lys Phe Lys Asn Asp Phe Arg
    790                 795                 800

TTA CAG CCA AGT TCT ACT TCT GAA TCT ATG GAT CAA CTA CTA AAC AAA      2622
Leu Gln Pro Ser Ser Thr Ser Glu Ser Met Asp Gln Leu Leu Asn Lys
805                 810                 815                 820

AAT AGA GAG GGA GAA AAA TCA AGA GAT TTG ATC AAA GAC AAA ATT GAA      2670
Asn Arg Glu Gly Glu Lys Ser Arg Asp Leu Ile Lys Asp Lys Ile Glu
                825                 830                 835

CCA AGT GCT AAG GAT TCT TTC ATT GAA AAT AGC AGC AGC AAC TGT ACC      2718
Pro Ser Ala Lys Asp Ser Phe Ile Glu Asn Ser Ser Ser Asn Cys Thr
            840                 845                 850

AGT GGC AGC AGC AAG CCG AAT AGC CCC AGC ATT TCC CCT TCA ATA CTT      2766
Ser Gly Ser Ser Lys Pro Asn Ser Pro Ser Ile Ser Pro Ser Ile Leu
        855                 860                 865

AGT AAC ACG GAG CAC AAG AGG GGA CCT GAG GTC ACT TCC CAA GGG GTT      2814
Ser Asn Thr Glu His Lys Arg Gly Pro Glu Val Thr Ser Gln Gly Val
    870                 875                 880

CAG ACT TCC AGC CCA GCA TGT AAA CAA GAG AAA GAC GAT AAG GAA GAG      2862
Gln Thr Ser Ser Pro Ala Cys Lys Gln Glu Lys Asp Asp Lys Glu Glu
885                 890                 895                 900

AAG AAA GAC GCA GCT GAG CAA GTT AGG AAA TCA ACA TTG AAT CCC AAT      2910
Lys Lys Asp Ala Ala Glu Gln Val Arg Lys Ser Thr Leu Asn Pro Asn
                905                 910                 915

GCA AAG GAG TTC AAC CCA CGT TCC TTC TCT CAG CCA AAG CCT TCT ACT      2958
Ala Lys Glu Phe Asn Pro Arg Ser Phe Ser Gln Pro Lys Pro Ser Thr
            920                 925                 930

ACC CCA ACT TCA CCT CGG CCT CAA GCA CAA CCT AGC CCA TCT ATG GTG      3006
Thr Pro Thr Ser Pro Arg Pro Gln Ala Gln Pro Ser Pro Ser Met Val
        935                 940                 945

GGT CAT CAA CAG CCA ACT CCA GTT TAT ACT CAG CCT GTT TGT TTT GCA      3054
Gly His Gln Gln Pro Thr Pro Val Tyr Thr Gln Pro Val Cys Phe Ala
    950                 955                 960
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CCA AAT ATG ATG TAT CCA GTC CCA GTG AGC CCA GGC GTG CAA CCT TTA      3102
Pro Asn Met Met Tyr Pro Val Pro Val Ser Pro Gly Val Gln Pro Leu
965                 970                 975                 980

TAC CCA ATA CCT ATG ACG CCC ATG CCA GTG AAT CAA GCC AAG ACA TAT      3150
Tyr Pro Ile Pro Met Thr Pro Met Pro Val Asn Gln Ala Lys Thr Tyr
                985                 990                 995

AGA GCA GTA CCA AAT ATG CCC CAA CAG CGG CAA GAC CAG CAT CAT CAG      3198
Arg Ala Val Pro Asn Met Pro Gln Gln Arg Gln Asp Gln His His Gln
            1000                1005                1010

AGT GCC ATG ATG CAC CCA GCG TCA GCA GCG GGC CCA CCG ATT GCA GCC      3246
Ser Ala Met Met His Pro Ala Ser Ala Ala Gly Pro Pro Ile Ala Ala
        1015                1020                1025

ACC CCA CCA GCT TAC TCC ACG CAA TAT GTT GCC TAC AGT CCT CAG CAG      3294
Thr Pro Pro Ala Tyr Ser Thr Gln Tyr Val Ala Tyr Ser Pro Gln Gln
    1030                1035                1040

TTC CCA AAT CAG CCC CTT GTT CAG CAT GTG CCA CAT TAT CAG TCT CAG      3342
Phe Pro Asn Gln Pro Leu Val Gln His Val Pro His Tyr Gln Ser Gln
1045                1050                1055                1060

CAT CCT CAT GTC TAT AGT CCT GTA ATA CAG GGT AAT GCT AGA ATG ATG      3390
His Pro His Val Tyr Ser Pro Val Ile Gln Gly Asn Ala Arg Met Met
                1065                1070                1075

GCA CCA CCA ACA CAC GCC CAG CCT GGT TTA GTA TCT TCT TCA GCA ACT      3438
Ala Pro Pro Thr His Ala Gln Pro Gly Leu Val Ser Ser Ser Ala Thr
            1080                1085                1090

CAG TAC GGG GCT CAT GAG CAG ACG CAT GCG ATG TAT GCA TGT CCC AAA      3486
Gln Tyr Gly Ala His Glu Gln Thr His Ala Met Tyr Ala Cys Pro Lys
        1095                1100                1105

TTA CCA TAC AAC AAG GAG ACA AGC CCT TCT TTC TAC TTT GCC ATT TCC      3534
Leu Pro Tyr Asn Lys Glu Thr Ser Pro Ser Phe Tyr Phe Ala Ile Ser
    1110                1115                1120

ACG GGC TCC CTT GCT CAG CAG TAT GCG CAC CCT AAC GCT ACC CTG CAC      3582
Thr Gly Ser Leu Ala Gln Gln Tyr Ala His Pro Asn Ala Thr Leu His
1125                1130                1135                1140

CCA CAT ACT CCA CAC CCT CAG CCT TCA GCT ACC CCC ACT GGA CAG CAG      3630
Pro His Thr Pro His Pro Gln Pro Ser Ala Thr Pro Thr Gly Gln Gln
                1145                1150                1155

CAA AGC CAA CAT GGT GGA AGT CAT CCT GCA CCC AGT CCT GTT CAG CAC      3678
Gln Ser Gln His Gly Gly Ser His Pro Ala Pro Ser Pro Val Gln His
            1160                1165                1170

CAT CAG CAC CAG GCC GCC CAG GCT CTC CAT CTG GCC AGT CCA CAG CAG      3726
His Gln His Gln Ala Ala Gln Ala Leu His Leu Ala Ser Pro Gln Gln
        1175                1180                1185
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CAG TCA GCC ATT TAC CAC GCG GGG CTT GCG CCA ACT CCA CCC TCC ATG      3774
Gln Ser Ala Ile Tyr His Ala Gly Leu Ala Pro Thr Pro Pro Ser Met
    1190                1195                1200

ACA CCT GCC TCC AAC ACG CAG TCG CCA CAG AAT AGT TTC CCA GCA GCA      3822
Thr Pro Ala Ser Asn Thr Gln Ser Pro Gln Asn Ser Phe Pro Ala Ala
1205                1210                1215                1220

CAA CAG ACT GTC TTT ACG ATC CAT CCT TCT CAC GTT CAG CCG GCG TAT      3870
Gln Gln Thr Val Phe Thr Ile His Pro Ser His Val Gln Pro Ala Tyr
                1225                1230                1235

ACC AAC CCA CCC CAC ATG GCC CAC GTA CCT CAG GCT CAT GTA CAG TCA      3918
Thr Asn Pro Pro His Met Ala His Val Pro Gln Ala His Val Gln Ser
            1240                1245                1250

GGA ATG GTT CCT TCT CAT CCA ACT GCC CAT GCG CCA ATG ATG CTA ATG      3966
Gly Met Val Pro Ser His Pro Thr Ala His Ala Pro Met Met Leu Met
        1255                1260                1265

ACG ACA CAG CCA CCC GGC GGT CCC CAG GCC GCC CTC GCT CAA AGT GCA      4014
Thr Thr Gln Pro Pro Gly Gly Pro Gln Ala Ala Leu Ala Gln Ser Ala
    1270                1275                1280

CTA CAG CCC ATT CCA GTC TCG ACA ACA GCG CAT TTC CCC TAT ATG ACG      4062
Leu Gln Pro Ile Pro Val Ser Thr Thr Ala His Phe Pro Tyr Met Thr
1285                1290                1295                1300

CAC CCT TCA GTA CAA GCC CAC CAC CAA CAG CAG TTG TAAGGCTGCC           4108
His Pro Ser Val Gln Ala His His Gln Gln Gln Leu
                1305                1310

CTGGAGGAAC CGAAAGGCCA AATTCCCTCC TCCCTTCTAC TGCTTCTACC AACTGGAAGC   4168

ACAGAAAACT AGAATTTCAT TTATTTTGTT TTTAAAATAT ATATGTTGAT TTCTTGTAAC   4228

ATCCAATAGG AATGCTAACA GTTCACTTGC AGTGGAAGAT ACTTGGACCG AGTAGAGGCA   4288

TTTAGGAACT TGGGGGCTAT TCCATAATTC CATATGCTGT TTCAGAGTCC CGCAGGTACC   4348

CCAGCTCTGC TTGCCGAAAC TGGAAGTTAT TTATTTTTTA ATAACCCTTG AAAGTCATGA   4408

ACACATCAGC TAGCAAAAGA AGTAACAAGA GTGATTCTTG CTGCTATTAC TGCTAAAAAA   4468

AAAAAAAAAA AAA                                                      4481
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1312 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Arg Ser Ala Ala Ala Ala Pro Arg Ser Pro Ala Val Ala Thr Glu
1 5 10 15

Ser Arg Arg Phe Ala Ala Ala Arg Trp Pro Gly Trp Arg Ser Leu Gln
20 25 30

Arg Pro Ala Arg Arg Ser Gly Arg Gly Gly Gly Gly Ala Ala Pro Gly
35 40 45

Pro Tyr Pro Ser Ala Ala Pro Pro Pro Pro Gly Pro Gly Pro Pro Pro
50 55 60

Ser Arg Gln Ser Ser Pro Pro Ser Ala Ser Asp Cys Phe Gly Ser Asn
65 70 75 80

Gly Asn Gly Gly Gly Ala Phe Arg Pro Gly Ser Arg Arg Leu Leu Gly
85 90 95

Leu Gly Gly Pro Pro Arg Pro Phe Val Val Val Leu Leu Pro Leu Ala
100 105 110

Ser Pro Gly Ala Pro Pro Ala Ala Pro Thr Arg Ala Ser Pro Leu Gly
115 120 125

Ala Arg Ala Ser Pro Pro Arg Ser Gly Val Ser Leu Ala Arg Pro Ala
130 135 140

Pro Gly Cys Pro Arg Pro Ala Cys Glu Pro Val Tyr Gly Pro Leu Thr
145 150 155 160

Met Ser Leu Lys Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
165 170 175

Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Pro Pro Pro Ala Ala
180 185 190

Ala Asn Val Arg Lys Pro Gly Gly Ser Gly Leu Leu Ala Ser Pro Ala
195 200 205

Ala Ala Pro Ser Pro Ser Ser Ser Ser Val Ser Ser Ser Ser Ala Thr
210 215 220

Ala Pro Ser Ser Val Val Ala Ala Thr Ser Gly Gly Gly Arg Pro Gly
225 230 235 240

Leu Gly Arg Gly Arg Asn Ser Asn Lys Gly Leu Pro Gln Ser Thr Ile
245 250 255

Ser Phe Asp Gly Ile Tyr Ala Asn Met Arg Met Val His Ile Leu Thr
260 265 270

Ser Val Val Gly Ser Lys Cys Glu Val Gln Val Lys Asn Gly Gly Ile
275 280 285

Tyr Glu Gly Val Phe Lys Thr Tyr Ser Pro Lys Cys Asp Leu Val Leu
290 295 300

Asp Ala Ala His Glu Lys Ser Thr Glu Ser Ser Ser Gly Pro Lys Arg
305 310 315 320

Glu Glu Ile Met Glu Ser Ile Leu Phe Lys Cys Ser Asp Phe Val Val
325 330 335

Val Gln Phe Lys Asp Met Asp Ser Ser Tyr Ala Lys Arg Asp Ala Phe
340 345 350

Thr Asp Ser Ala Ile Ser Ala Lys Val Asn Gly Glu His Lys Glu Lys
355 360 365

Asp Leu Glu Pro Trp Asp Ala Gly Glu Leu Thr Ala Asn Glu Glu Leu
370 375 380

Glu Ala Leu Glu Asn Asp Val Ser Asn Gly Trp Asp Pro Asn Asp Met
385 390 395 400

Phe Arg Tyr Asn Glu Glu Asn Tyr Gly Val Val Ser Thr Tyr Asp Ser
405 410 415

Ser Leu Ser Ser Tyr Thr Val Pro Leu Glu Arg Asp Asn Ser Glu Glu
420 425 430

Phe Leu Lys Arg Glu Ala Arg Ala Asn Gln Leu Ala Glu Glu Ile Glu
435 440 445

Ser Ser Ala Gln Tyr Lys Ala Arg Val Ala Leu Glu Asn Asp Asp Arg
450 455 460

Ser Glu Glu Glu Lys Tyr Thr Ala Val Gln Arg Asn Ser Ser Glu Arg
465 470 475 480

Glu Gly His Ser Ile Asn Thr Arg Glu Asn Lys Tyr Ile Pro Pro Gly
485 490 495

Gln Arg Asn Arg Glu Val Ile Ser Trp Gly Ser Gly Arg Gln Asn Ser
500 505 510

Pro Arg Met Gly Gln Pro Gly Ser Gly Ser Met Pro Ser Arg Ser Thr
515 520 525

Ser His Thr Ser Asp Phe Asn Pro Asn Ser Gly Ser Asp Gln Arg Val
530 535 540

Val Asn Gly Gly Val Pro Trp Pro Ser Pro Cys Pro Ser Pro Ser Ser
545 550 555 560

Arg Pro Pro Ser Arg Tyr Gln Ser Gly Pro Asn Ser Leu Pro Pro Arg
565 570 575

Ala Ala Thr Pro Thr Arg Pro Pro Ser Arg Pro Pro Ser Arg Pro Ser
580 585 590

Arg Pro Pro Ser His Pro Ser Ala His Gly Ser Pro Ala Pro Val Ser
595 600 605

Thr Met Pro Lys Arg Met Ser Ser Glu Gly Pro Pro Arg Met Ser Pro
610 615 620

Lys Ala Gln Arg His Pro Arg Asn His Arg Val Ser Ala Gly Arg Gly
625 630 635 640

Ser Ile Ser Ser Gly Leu Glu Phe Val Ser His Asn Pro Pro Ser Glu
645 650 655

Ala Ala Thr Pro Pro Val Ala Arg Thr Ser Pro Ser Gly Gly Thr Trp
660 665 670

Ser Ser Val Val Ser Gly Val Pro Arg Leu Ser Pro Lys Thr His Arg
675 680 685

Pro Arg Ser Pro Arg Gln Asn Ser Ile Gly Asn Thr Pro Ser Gly Pro
690 695 700

Val Leu Ala Ser Pro Gln Ala Gly Ile Ile Pro Thr Glu Ala Val Ala
705 710 715 720

Met Pro Ile Pro Ala Ala Ser Pro Thr Pro Ala Ser Pro Ala Ser Asn
725 730 735

Arg Ala Val Thr Pro Ser Ser Glu Ala Lys Asp Ser Arg Leu Gln Asp
740 745 750

Gln Arg Gln Asn Ser Pro Ala Gly Asn Lys Glu Asn Ile Lys Pro Asn
755 760 765

Glu Thr Ser Pro Ser Phe Ser Lys Ala Glu Asn Lys Gly Ile Ser Pro
770 775 780

Val Val Ser Glu His Arg Lys Gln Ile Asp Asp Leu Lys Lys Phe Lys
785 790 795 800

Asn Asp Phe Arg Leu Gln Pro Ser Ser Thr Ser Glu Ser Met Asp Gln
805 810 815

Leu Leu Asn Lys Asn Arg Glu Gly Glu Lys Ser Arg Asp Leu Ile Lys
820 825 830

Asp Lys Ile Glu Pro Ser Ala Lys Asp Ser Phe Ile Glu Asn Ser Ser
835 840 845

Ser Asn Cys Thr Ser Gly Ser Ser Lys Pro Asn Ser Pro Ser Ile Ser
850 855 860

Pro Ser Ile Leu Ser Asn Thr Glu His Lys Arg Gly Pro Glu Val Thr
865 870 875 880

Ser Gln Gly Val Gln Thr Ser Ser Pro Ala Cys Lys Gln Glu Lys Asp
885 890 895

Asp Lys Glu Glu Lys Lys Asp Ala Ala Glu Gln Val Arg Lys Ser Thr
900 905 910

Leu Asn Pro Asn Ala Lys Glu Phe Asn Pro Arg Ser Phe Ser Gln Pro
915 920 925

Lys Pro Ser Thr Thr Pro Thr Ser Pro Arg Pro Gln Ala Gln Pro Ser
930 935 940

Pro Ser Met Val Gly His Gln Gln Pro Thr Pro Val Tyr Thr Gln Pro
945 950 955 960

Val Cys Phe Ala Pro Asn Met Met Tyr Pro Val Pro Val Ser Pro Gly
965 970 975

Val Gln Pro Leu Tyr Pro Ile Pro Met Thr Pro Met Pro Val Asn Gln
980 985 990

Ala Lys Thr Tyr Arg Ala Val Pro Asn Met Pro Gln Gln Arg Gln Asp
995 1000 1005

Gln His His Gln Ser Ala Met Met His Pro Ala Ser Ala Ala Gly Pro
1010 1015 1020

Pro Ile Ala Ala Thr Pro Pro Ala Tyr Ser Thr Gln Tyr Val Ala Tyr
1025 1030 1035 1040

Ser Pro Gln Gln Phe Pro Asn Gln Pro Leu Val Gln His Val Pro His
1045 1050 1055

Tyr Gln Ser Gln His Pro His Val Tyr Ser Pro Val Ile Gln Gly Asn
1060 1065 1070

Ala Arg Met Met Ala Pro Pro Thr His Ala Gln Pro Gly Leu Val Ser
1075 1080 1085

Ser Ser Ala Thr Gln Tyr Gly Ala His Glu Gln Thr His Ala Met Tyr
1090 1095 1100

Ala Cys Pro Lys Leu Pro Tyr Asn Lys Glu Thr Ser Pro Ser Phe Tyr
1105 1110 1115 1120

Phe Ala Ile Ser Thr Gly Ser Leu Ala Gln Gln Tyr Ala His Pro Asn
1125 1130 1135

Ala Thr Leu His Pro His Thr Pro His Pro Gln Pro Ser Ala Thr Pro
1140 1145 1150

Thr Gly Gln Gln Gln Ser Gln His Gly Gly Ser His Pro Ala Pro Ser
1155 1160 1165

Pro Val Gln His His Gln His Gln Ala Ala Gln Ala Leu His Leu Ala
1170 1175 1180

Ser Pro Gln Gln Gln Ser Ala Ile Tyr His Ala Gly Leu Ala Pro Thr
1185 1190 1195 1200

Pro Pro Ser Met Thr Pro Ala Ser Asn Thr Gln Ser Pro Gln Asn Ser
1205 1210 1215

Phe Pro Ala Ala Gln Gln Thr Val Phe Thr Ile His Pro Ser His Val
1220 1225 1230

Gln Pro Ala Tyr Thr Asn Pro Pro His Met Ala His Val Pro Gln Ala
1235 1240 1245

His Val Gln Ser Gly Met Val Pro Ser His Pro Thr Ala His Ala Pro
1250 1255 1260

Met Met Leu Met Thr Thr Gln Pro Pro Gly Gly Pro Gln Ala Ala Leu
1265 1270 1275 1280

Ala Gln Ser Ala Leu Gln Pro Ile Pro Val Ser Thr Thr Ala His Phe
1285 1290 1295

Pro Tyr Met Thr His Pro Ser Val Gln Ala His His Gln Gln Gln Leu
1300 1305 1310

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3798 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 50..3457

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGCACGAGGT CCCCGCCCGG CGTGCGAGCC GGTGTATGGG CCGCTCACC ATG TCG
Met Ser
1

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CTG AAG CCG CAG CCG CAG CCG CCC GCG CCC GCC ACT GGC CGC AAG CCC        103
Leu Lys Pro Gln Pro Gln Pro Pro Ala Pro Ala Thr Gly Arg Lys Pro
  5                  10                  15

GGC GGC GGC CTG CTC TCG TCG CCC GGC GCC GCG CCG GCC TCG GCC GCG        151
Gly Gly Gly Leu Leu Ser Ser Pro Gly Ala Ala Pro Ala Ser Ala Ala
 20                  25                  30

GTG ACC TCG GCT TCC GTG GTG CCG GCC CCG GCC GCG CCG GTG GCG TCT        199
Val Thr Ser Ala Ser Val Val Pro Ala Pro Ala Ala Pro Val Ala Ser
 35                  40                  45                  50

TCC TCG GCG GCC GCG GGC GGC GGG CGT CCC GGC CTG GGC AGA GGT CGG        247
Ser Ser Ala Ala Ala Gly Gly Gly Arg Pro Gly Leu Gly Arg Gly Arg
                 55                  60                  65

AAC AGT AGC AAA GGA CTG CCT CAG CCT ACG ATT TCT TTT GAT GGA ATC        295
Asn Ser Ser Lys Gly Leu Pro Gln Pro Thr Ile Ser Phe Asp Gly Ile
             70                  75                  80

TAT GCA AAC GTG AGG ATG GTT CAT ATA CTT ACG TCA GTT GTT GGA TCG        343
Tyr Ala Asn Val Arg Met Val His Ile Leu Thr Ser Val Val Gly Ser
         85                  90                  95

AAA TGT GAA GTA CAA GTG AAA AAC GGA GGC ATA TAT GAA GGA GTT TTT        391
Lys Cys Glu Val Gln Val Lys Asn Gly Gly Ile Tyr Glu Gly Val Phe
    100                 105                 110

AAA ACA TAC AGT CCT AAG TGT GAC TTG GTA CTT GAT GCT GCA CAT GAG        439
Lys Thr Tyr Ser Pro Lys Cys Asp Leu Val Leu Asp Ala Ala His Glu
115                 120                 125                 130

AAA AGT ACA GAA TCC AGT TCG GGG CCA AAA CGT GAA GAA ATA ATG GAG        487
Lys Ser Thr Glu Ser Ser Ser Gly Pro Lys Arg Glu Glu Ile Met Glu
                135                 140                 145

AGT GTT TTG TTC AAA TGC TCA GAC TTC GTT GTG GTA CAG TTT AAA GAT        535
Ser Val Leu Phe Lys Cys Ser Asp Phe Val Val Val Gln Phe Lys Asp
            150                 155                 160

ACA GAC TCC AGT TAT GCA CGG AGA GAT GCT TTT ACT GAC TCT GCT CTC        583
Thr Asp Ser Ser Tyr Ala Arg Arg Asp Ala Phe Thr Asp Ser Ala Leu
        165                 170                 175

AGC GCA AAG GTG AAT GGT GAG CAC AAG GAG AAG GAC CTG GAG CCC TGG        631
Ser Ala Lys Val Asn Gly Glu His Lys Glu Lys Asp Leu Glu Pro Trp
    180                 185                 190

GAT GCA GGG GAG CTC ACG GCC AGC GAG GAG CTG GAG CTG GAG AAT GAT        679
Asp Ala Gly Glu Leu Thr Ala Ser Glu Glu Leu Glu Leu Glu Asn Asp
195                 200                 205                 210

GTG TCT AAT GGA TGG GAC CCC AAT GAC ATG TTT CGA TAT AAT GAA GAG        727
Val Ser Asn Gly Trp Asp Pro Asn Asp Met Phe Arg Tyr Asn Glu Glu
                215                 220                 225
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AAT TAT GGT GTG GTG TCC ACA TAT GAT AGC AGT TTA TCT TCA TAT ACG      775
Asn Tyr Gly Val Val Ser Thr Tyr Asp Ser Ser Leu Ser Ser Tyr Thr
            230                 235                 240

GTT CCT TTA GAA AGG GAC AAC TCA GAA GAA TTT CTT AAA CGG GAG GCA      823
Val Pro Leu Glu Arg Asp Asn Ser Glu Glu Phe Leu Lys Arg Glu Ala
        245                 250                 255

AGG GCA AAC CAG TTA GCA GAA GAA ATT GAA TCC AGT GCT CAG TAC AAA      871
Arg Ala Asn Gln Leu Ala Glu Glu Ile Glu Ser Ser Ala Gln Tyr Lys
    260                 265                 270

GCT CGT GTC GCC CTT GAG AAT GAT GAC CGG AGT GAG GAA GAA AAA TAC      919
Ala Arg Val Ala Leu Glu Asn Asp Asp Arg Ser Glu Glu Glu Lys Tyr
275                 280                 285                 290

ACA GCA GTC CAG AGA AAC TGC AGT GAC CGG GAG GGG CAT GGC CCC AAC      967
Thr Ala Val Gln Arg Asn Cys Ser Asp Arg Glu Gly His Gly Pro Asn
                295                 300                 305

ACT AGG GAC AAT AAA TAT ATT CCT CCT GGA CAA AGA AAC AGA GAA GTC     1015
Thr Arg Asp Asn Lys Tyr Ile Pro Pro Gly Gln Arg Asn Arg Glu Val
            310                 315                 320

CTA TCC TGG GGA AGT GGG AGA CAG AGC TCA CCA CGG ATG GGC CAG CCT     1063
Leu Ser Trp Gly Ser Gly Arg Gln Ser Ser Pro Arg Met Gly Gln Pro
        325                 330                 335

GGG CCA GGC TCC ATG CCG TCA AGA GCT GCT TCT CAC ACT TCA GAT TTC     1111
Gly Pro Gly Ser Met Pro Ser Arg Ala Ala Ser His Thr Ser Asp Phe
    340                 345                 350

AAC CCG AAC GCT GGC TCA GAC CAA AGA GTA GTT AAT GGA GGT GTT CCC     1159
Asn Pro Asn Ala Gly Ser Asp Gln Arg Val Val Asn Gly Gly Val Pro
355                 360                 365                 370

TGG CCA TCG CCT TGC CCA TCT CAT TCC TCT CGC CCA CCT TCT CGC TAC     1207
Trp Pro Ser Pro Cys Pro Ser His Ser Ser Arg Pro Pro Ser Arg Tyr
                375                 380                 385

CAG TCA GGT CCC AAC TCT CTT CCA CCT CGG GCA GCC ACC CAT ACA CGG     1255
Gln Ser Gly Pro Asn Ser Leu Pro Pro Arg Ala Ala Thr His Thr Arg
            390                 395                 400

CCG CCC TCC AGG CCC CCC TCG AGG CCA TCC AGA CCC CCG TCT CAC CCC     1303
Pro Pro Ser Arg Pro Pro Ser Arg Pro Ser Arg Pro Pro Ser His Pro
        405                 410                 415

TCT GCT CAT GGT TCT CCA GCT CCT GTC TCT ACT ATG CCT AAA CGC ATG     1351
Ser Ala His Gly Ser Pro Ala Pro Val Ser Thr Met Pro Lys Arg Met
    420                 425                 430

TCT TCA GAA GGA CCC CCA AGG ATG TCT CCA AAG GCA CAG CGC CAC CCT     1399
Ser Ser Glu Gly Pro Pro Arg Met Ser Pro Lys Ala Gln Arg His Pro
435                 440                 445                 450
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CGG AAT CAC AGA GTC TCT GCT GGG AGA GGC TCC ATG TCT AGT GGC CTA      1447
Arg Asn His Arg Val Ser Ala Gly Arg Gly Ser Met Ser Ser Gly Leu
                455                 460                 465

GAA TTT GTA TCC CAC AAT CCC CCA AGT GAA GCA GCT GCT CCT CCA GTG      1495
Glu Phe Val Ser His Asn Pro Pro Ser Glu Ala Ala Ala Pro Pro Val
            470                 475                 480

GCA AGG ACC AGT CCT GCA GGG GGA ACG TGG TCC TCA GTG GTC AGT GGG      1543
Ala Arg Thr Ser Pro Ala Gly Gly Thr Trp Ser Ser Val Val Ser Gly
        485                 490                 495

GTT CCA AGG TTA TCT CCC AAA ACT CAC AGA CCC AGG TCT CCC AGG CAG      1591
Val Pro Arg Leu Ser Pro Lys Thr His Arg Pro Arg Ser Pro Arg Gln
    500                 505                 510

AGC AGC ATT GGA AAC TCT CCC AGC GGG CCT GTG CTT GCT TCT CCC CAA      1639
Ser Ser Ile Gly Asn Ser Pro Ser Gly Pro Val Leu Ala Ser Pro Gln
515                 520                 525                 530

GCT GGC ATC ATC CCT GCA GAA GCC GTT TCC ATG CCT GTT CCC GCC GCA      1687
Ala Gly Ile Ile Pro Ala Glu Ala Val Ser Met Pro Val Pro Ala Ala
                535                 540                 545

TCT CCG ACT CCT GCC AGC CCT GCA TCC AAC AGA GCA CTG ACC CCA TCT      1735
Ser Pro Thr Pro Ala Ser Pro Ala Ser Asn Arg Ala Leu Thr Pro Ser
            550                 555                 560

ATT GAG GCA AAA GAT TCC AGG CTT CAA GAT CAG AGG CAG AAC TCT CCT      1783
Ile Glu Ala Lys Asp Ser Arg Leu Gln Asp Gln Arg Gln Asn Ser Pro
        565                 570                 575

GCA GGG AGT AAA GAA AAT GTT AAA GCA AGT GAA ACA TCA CCT AGC TTT      1831
Ala Gly Ser Lys Glu Asn Val Lys Ala Ser Glu Thr Ser Pro Ser Phe
    580                 585                 590

TCA AAA GCT GAC AAC AAA GGT ATG TCA CCA GTT GTT TCT GAA CAC AGA      1879
Ser Lys Ala Asp Asn Lys Gly Met Ser Pro Val Val Ser Glu His Arg
595                 600                 605                 610

AAA CAG ATT GAT GAC TTA AAG AAG TTT AAG AAT GAT TTT AGG TTA CAG      1927
Lys Gln Ile Asp Asp Leu Lys Lys Phe Lys Asn Asp Phe Arg Leu Gln
                615                 620                 625

CCA AGC TCT ACA TCT GAA TCT ATG GAT CAA CTA CTA AGC AAA AAT AGA      1975
Pro Ser Ser Thr Ser Glu Ser Met Asp Gln Leu Leu Ser Lys Asn Arg
            630                 635                 640

GAA GGA GAA AAG TCA CGA GAT TTG ATT AAA GAT AAA ACG GAA GCA AGT      2023
Glu Gly Glu Lys Ser Arg Asp Leu Ile Lys Asp Lys Thr Glu Ala Ser
        645                 650                 655

GCT AAG GAT AGT TTC ATT GAC AGC AGC AGC AGC AGC AGC AAC TGT ACC      2071
Ala Lys Asp Ser Phe Ile Asp Ser Ser Ser Ser Ser Ser Asn Cys Thr
    660                 665                 670
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AGT GGC AGC AGC AAG ACC AAC AGC CCT AGC ATC TCC CCT TCC ATG CTT      2119
Ser Gly Ser Ser Lys Thr Asn Ser Pro Ser Ile Ser Pro Ser Met Leu
675                 680                 685                 690

AGT AAT GCA GAG CAC AAG AGG GGG CCT GAG GTC ACA TCC CAA GGG GTG      2167
Ser Asn Ala Glu His Lys Arg Gly Pro Glu Val Thr Ser Gln Gly Val
                695                 700                 705

CAG ACT TCC AGC CCA GCC TGC AAA CAA GAG AAG GAT GAC AGA GAA GAG      2215
Gln Thr Ser Ser Pro Ala Cys Lys Gln Glu Lys Asp Asp Arg Glu Glu
            710                 715                 720

AAG AAA GAC ACA ACA GAG CAG GTT AGG AAA TCG ACA TTG AAT CCC AAT      2263
Lys Lys Asp Thr Thr Glu Gln Val Arg Lys Ser Thr Leu Asn Pro Asn
        725                 730                 735

GCA AAG GAG TTC AAC CCT CGT TCT TTC TCT CAG CCA AAG CCT TCT ACT      2311
Ala Lys Glu Phe Asn Pro Arg Ser Phe Ser Gln Pro Lys Pro Ser Thr
    740                 745                 750

ACC CCA ACG TCA CCT CGG CCT CAA GCA CAA CCC AGC CCA TCT ATG GTG      2359
Thr Pro Thr Ser Pro Arg Pro Gln Ala Gln Pro Ser Pro Ser Met Val
755                 760                 765                 770

GGT CAT CAG CAG CCA GCT CCA GTG TAC ACT CAG CCT GTG TGC TTC GCA      2407
Gly His Gln Gln Pro Ala Pro Val Tyr Thr Gln Pro Val Cys Phe Ala
                775                 780                 785

CCC AAT ATG ATG TAT CCC GTC CCA GTG AGC CCG GGC GTA CAA CCT TTA      2455
Pro Asn Met Met Tyr Pro Val Pro Val Ser Pro Gly Val Gln Pro Leu
            790                 795                 800

TAC CCA ATA CCT ATG ACG CCC ATG CCT GTG AAC CAA GCC AAG ACA TAT      2503
Tyr Pro Ile Pro Met Thr Pro Met Pro Val Asn Gln Ala Lys Thr Tyr
        805                 810                 815

AGA GCA GGT AAA GTA CCA AAT ATG CCC CAA CAG CGA CAA GAC CAA CAT      2551
Arg Ala Gly Lys Val Pro Asn Met Pro Gln Gln Arg Gln Asp Gln His
    820                 825                 830

CAT CAA AGC ACC ATG ATG CAC CCA GCC TCC GCG GCA GGG CCA CCC ATC      2599
His Gln Ser Thr Met Met His Pro Ala Ser Ala Ala Gly Pro Pro Ile
835                 840                 845                 850

GTA GCC ACC CCG CCC GCT TAC TCC ACT CAG TAC GTT GCC TAC AGC CCT      2647
Val Ala Thr Pro Pro Ala Tyr Ser Thr Gln Tyr Val Ala Tyr Ser Pro
                855                 860                 865

CAG CAG TTT CCC AAT CAG CCT TTG GTC CAG CAT GTG CCG CAT TAT CAG      2695
Gln Gln Phe Pro Asn Gln Pro Leu Val Gln His Val Pro His Tyr Gln
            870                 875                 880

TCT CAG CAT CCT CAT GTG TAC AGT CCT GTC ATA CAA GGT AAT GCC AGG      2743
Ser Gln His Pro His Val Tyr Ser Pro Val Ile Gln Gly Asn Ala Arg
        885                 890                 895
```

```
ATG ATG GCA CCA CCA GCA CAT GCT CAG CCT GGT TTA GTG TCT TCT TCA      2791
Met Met Ala Pro Pro Ala His Ala Gln Pro Gly Leu Val Ser Ser Ser
    900                 905                 910

GCT GCT CAG TTC GGG GCT CAC GAG CAG ACG CAC GCC ATG TAT GCA TGT      2839
Ala Ala Gln Phe Gly Ala His Glu Gln Thr His Ala Met Tyr Ala Cys
915                 920                 925                 930

CCC AAA TTA CCA TAC AAC AAG GAG ACA AGC CCT TCT TTC TAC TTT GCC      2887
Pro Lys Leu Pro Tyr Asn Lys Glu Thr Ser Pro Ser Phe Tyr Phe Ala
                935                 940                 945

ATT TCC ACC GGC TCC CTC GCT CAG CAG TAT GCA CAT CCT AAT GCC GCC      2935
Ile Ser Thr Gly Ser Leu Ala Gln Gln Tyr Ala His Pro Asn Ala Ala
            950                 955                 960

CTG CAT CCA CAT ACT CCC CAT CCT CAG CCT TCG GCC ACT CCC ACC GGA      2983
Leu His Pro His Thr Pro His Pro Gln Pro Ser Ala Thr Pro Thr Gly
        965                 970                 975

CAG CAG CAA AGC CAG CAT GGT GGA AGT CAC CCT GCA CCC AGT CCT GTT      3031
Gln Gln Gln Ser Gln His Gly Gly Ser His Pro Ala Pro Ser Pro Val
    980                 985                 990

CAG CAC CAT CAG CAC CAG GCT GCC CAG GCT CTT CAT CTG GCC AGT CCA      3079
Gln His His Gln His Gln Ala Ala Gln Ala Leu His Leu Ala Ser Pro
995                 1000                1005                1010

CAG CAG CAG TCG GCC ATT TAT CAT GCG GGG CTG GCA CCA ACA CCA CCT      3127
Gln Gln Gln Ser Ala Ile Tyr His Ala Gly Leu Ala Pro Thr Pro Pro
                1015                1020                1025

TCC ATG ACA CCT GCC TCT AAT ACA CAG TCT CCA CAG AGC AGT TTC CCA      3175
Ser Met Thr Pro Ala Ser Asn Thr Gln Ser Pro Gln Ser Ser Phe Pro
            1030                1035                1040

GCA GCA CAA CAG ACA GTC TTC ACC ATC CAC CCT TCT CAT GTT CAG CCG      3223
Ala Ala Gln Gln Thr Val Phe Thr Ile His Pro Ser His Val Gln Pro
        1045                1050                1055

GCA TAC ACC ACC CCA CCC CAC ATG GCC CAC GTA CCT CAG GCT CAT GTA      3271
Ala Tyr Thr Thr Pro Pro His Met Ala His Val Pro Gln Ala His Val
    1060                1065                1070

CAG TCA GGA ATG GTT CCT TCT CAT CCA ACT GCC CAT GCG CCA ATG ATG      3319
Gln Ser Gly Met Val Pro Ser His Pro Thr Ala His Ala Pro Met Met
1075                1080                1085                1090

CTA ATG ACG ACA CAG CCA CCC GGT CCC AAG GCC GCC CTC GCT CAA AGT      3367
Leu Met Thr Thr Gln Pro Pro Gly Pro Lys Ala Ala Leu Ala Gln Ser
                1095                1100                1105

GCA CTA CAG CCC ATT CCA GTT TCG ACA ACA GCG CAT TTC CCT TAT ATG      3415
Ala Leu Gln Pro Ile Pro Val Ser Thr Thr Ala His Phe Pro Tyr Met
            1110                1115                1120
```

```
ACG CAC CCT TCA GTA CAA GCC CAC CAC CAA CAG CAG TTG TAAGGCTGCC      3464
Thr His Pro Ser Val Gln Ala His His Gln Gln Gln Leu
        1125                1130                1135

TTGGAGGAAC CGAAAGGCCA AATCCCTTCT TCCCTTCTCT GCTTCTGCCA ACCGGAAGCA  3524

CAGAAAACTA GAACTTCATT GATTTTGTTT TTTAAAAGAT ACACTGATTT AACATCTGAT  3584

AGGAATGCTA ACAGCTCACT TGCAGTGGAG GATGTTTTGG ACCGAGTAGA GGCATGTAGG  3644

GACTTGTGGC TGTTCCATAA TTCCATGTGC TGTTGCAGGG TCCTGCAAGT ACCCAGCTCT  3704

GCTTGCTGAA ACTGGAAGTT ATTTATTTTT TAATGGCCCT TGAGAGTCAT GAACACATCA  3764

GCTAGCAACA GAAGTAACAA GAGTGATTCT TGCT                               3798
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ser Leu Lys Pro Gln Pro Gln Pro Pro Ala Pro Ala Thr Gly Arg
 1               5                  10                  15

Lys Pro Gly Gly Gly Leu Leu Ser Ser Pro Gly Ala Ala Pro Ala Ser
            20                  25                  30

Ala Ala Val Thr Ser Ala Ser Val Val Pro Ala Pro Ala Ala Pro Val
        35                  40                  45

Ala Ser Ser Ser Ala Ala Ala Gly Gly Gly Arg Pro Gly Leu Gly Arg
    50                  55                  60

Gly Arg Asn Ser Ser Lys Gly Leu Pro Gln Pro Thr Ile Ser Phe Asp
65                  70                  75                  80

Gly Ile Tyr Ala Asn Val Arg Met Val His Ile Leu Thr Ser Val Val
                85                  90                  95

Gly Ser Lys Cys Glu Val Gln Val Lys Asn Gly Gly Ile Tyr Glu Gly
            100                 105                 110

Val Phe Lys Thr Tyr Ser Pro Lys Cys Asp Leu Val Leu Asp Ala Ala
        115                 120                 125

His Glu Lys Ser Thr Glu Ser Ser Ser Gly Pro Lys Arg Glu Glu Ile
    130                 135                 140
```

Met Glu Ser Val Leu Phe Lys Cys Ser Asp Phe Val Val Val Gln Phe
145 150 155 160

Lys Asp Thr Asp Ser Ser Tyr Ala Arg Arg Asp Ala Phe Thr Asp Ser
165 170 175

Ala Leu Ser Ala Lys Val Asn Gly Glu His Lys Glu Lys Asp Leu Glu
180 185 190

Pro Trp Asp Ala Gly Glu Leu Thr Ala Ser Glu Glu Leu Glu Leu Glu
195 200 205

Asn Asp Val Ser Asn Gly Trp Asp Pro Asn Asp Met Phe Arg Tyr Asn
210 215 220

Glu Glu Asn Tyr Gly Val Val Ser Thr Tyr Asp Ser Ser Leu Ser Ser
225 230 235 240

Tyr Thr Val Pro Leu Glu Arg Asp Asn Ser Glu Glu Phe Leu Lys Arg
245 250 255

Glu Ala Arg Ala Asn Gln Leu Ala Glu Glu Ile Glu Ser Ser Ala Gln
260 265 270

Tyr Lys Ala Arg Val Ala Leu Glu Asn Asp Asp Arg Ser Glu Glu Glu
275 280 285

Lys Tyr Thr Ala Val Gln Arg Asn Cys Ser Asp Arg Glu Gly His Gly
290 295 300

Pro Asn Thr Arg Asp Asn Lys Tyr Ile Pro Pro Gly Gln Arg Asn Arg
305 310 315 320

Glu Val Leu Ser Trp Gly Ser Gly Arg Gln Ser Ser Pro Arg Met Gly
325 330 335

Gln Pro Gly Pro Gly Ser Met Pro Ser Arg Ala Ala Ser His Thr Ser
340 345 350

Asp Phe Asn Pro Asn Ala Gly Ser Asp Gln Arg Val Val Asn Gly Gly
355 360 365

Val Pro Trp Pro Ser Pro Cys Pro Ser His Ser Ser Arg Pro Pro Ser
370 375 380

Arg Tyr Gln Ser Gly Pro Asn Ser Leu Pro Pro Arg Ala Ala Thr His
385 390 395 400

Thr Arg Pro Pro Ser Arg Pro Pro Ser Arg Pro Ser Arg Pro Pro Ser
405 410 415

His Pro Ser Ala His Gly Ser Pro Ala Pro Val Ser Thr Met Pro Lys
420 425 430

```
Arg Met Ser Ser Glu Gly Pro Pro Arg Met Ser Pro Lys Ala Gln Arg
        435                 440                 445

His Pro Arg Asn His Arg Val Ser Ala Gly Arg Gly Ser Met Ser Ser
    450                 455                 460

Gly Leu Glu Phe Val Ser His Asn Pro Pro Ser Glu Ala Ala Ala Pro
465                 470                 475                 480

Pro Val Ala Arg Thr Ser Pro Ala Gly Gly Thr Trp Ser Ser Val Val
                485                 490                 495

Ser Gly Val Pro Arg Leu Ser Pro Lys Thr His Arg Pro Arg Ser Pro
            500                 505                 510

Arg Gln Ser Ser Ile Gly Asn Ser Pro Ser Gly Pro Val Leu Ala Ser
        515                 520                 525

Pro Gln Ala Gly Ile Ile Pro Ala Glu Ala Val Ser Met Pro Val Pro
    530                 535                 540

Ala Ala Ser Pro Thr Pro Ala Ser Pro Ala Ser Asn Arg Ala Leu Thr
545                 550                 555                 560

Pro Ser Ile Glu Ala Lys Asp Ser Arg Leu Gln Asp Gln Arg Gln Asn
                565                 570                 575

Ser Pro Ala Gly Ser Lys Glu Asn Val Lys Ala Ser Glu Thr Ser Pro
            580                 585                 590

Ser Phe Ser Lys Ala Asp Asn Lys Gly Met Ser Pro Val Val Ser Glu
        595                 600                 605

His Arg Lys Gln Ile Asp Asp Leu Lys Lys Phe Lys Asn Asp Phe Arg
    610                 615                 620

Leu Gln Pro Ser Ser Thr Ser Glu Ser Met Asp Gln Leu Leu Ser Lys
625                 630                 635                 640

Asn Arg Glu Gly Glu Lys Ser Arg Asp Leu Ile Lys Asp Lys Thr Glu
                645                 650                 655

Ala Ser Ala Lys Asp Ser Phe Ile Asp Ser Ser Ser Ser Ser Ser Asn
            660                 665                 670

Cys Thr Ser Gly Ser Ser Lys Thr Asn Ser Pro Ser Ile Ser Pro Ser
        675                 680                 685

Met Leu Ser Asn Ala Glu His Lys Arg Gly Pro Glu Val Thr Ser Gln
    690                 695                 700

Gly Val Gln Thr Ser Ser Pro Ala Cys Lys Gln Glu Lys Asp Asp Arg
705                 710                 715                 720
```

Glu Glu Lys Lys Asp Thr Thr Glu Gln Val Arg Lys Ser Thr Leu Asn
725 730 735

Pro Asn Ala Lys Glu Phe Asn Pro Arg Ser Phe Ser Gln Pro Lys Pro
740 745 750

Ser Thr Thr Pro Thr Ser Pro Arg Pro Gln Ala Gln Pro Ser Pro Ser
755 760 765

Met Val Gly His Gln Gln Pro Ala Pro Val Tyr Thr Gln Pro Val Cys
770 775 780

Phe Ala Pro Asn Met Met Tyr Pro Val Pro Val Ser Pro Gly Val Gln
785 790 795 800

Pro Leu Tyr Pro Ile Pro Met Thr Pro Met Pro Val Asn Gln Ala Lys
805 810 815

Thr Tyr Arg Ala Gly Lys Val Pro Asn Met Pro Gln Gln Arg Gln Asp
820 825 830

Gln His His Gln Ser Thr Met Met His Pro Ala Ser Ala Ala Gly Pro
835 840 845

Pro Ile Val Ala Thr Pro Pro Ala Tyr Ser Thr Gln Tyr Val Ala Tyr
850 855 860

Ser Pro Gln Gln Phe Pro Asn Gln Pro Leu Val Gln His Val Pro His
865 870 875 880

Tyr Gln Ser Gln His Pro His Val Tyr Ser Pro Val Ile Gln Gly Asn
885 890 895

Ala Arg Met Met Ala Pro Pro Ala His Ala Gln Pro Gly Leu Val Ser
900 905 910

Ser Ser Ala Ala Gln Phe Gly Ala His Glu Gln Thr His Ala Met Tyr
915 920 925

Ala Cys Pro Lys Leu Pro Tyr Asn Lys Glu Thr Ser Pro Ser Phe Tyr
930 935 940

Phe Ala Ile Ser Thr Gly Ser Leu Ala Gln Gln Tyr Ala His Pro Asn
945 950 955 960

Ala Ala Leu His Pro His Thr Pro His Pro Gln Pro Ser Ala Thr Pro
965 970 975

Thr Gly Gln Gln Gln Ser Gln His Gly Gly Ser His Pro Ala Pro Ser
980 985 990

Pro Val Gln His His Gln His Gln Ala Ala Gln Ala Leu His Leu Ala
995 1000 1005

Ser Pro Gln Gln Gln Ser Ala Ile Tyr His Ala Gly Leu Ala Pro Thr
1010 1015 1020

Pro Pro Ser Met Thr Pro Ala Ser Asn Thr Gln Ser Pro Gln Ser Ser
1025 1030 1035 1040

Phe Pro Ala Ala Gln Gln Thr Val Phe Thr Ile His Pro Ser His Val
1045 1050 1055

Gln Pro Ala Tyr Thr Thr Pro Pro His Met Ala His Val Pro Gln Ala
1060 1065 1070

His Val Gln Ser Gly Met Val Pro Ser His Pro Thr Ala His Ala Pro
1075 1080 1085

Met Met Leu Met Thr Thr Gln Pro Pro Gly Pro Lys Ala Ala Leu Ala
1090 1095 1100

Gln Ser Ala Leu Gln Pro Ile Pro Val Ser Thr Thr Ala His Phe Pro
1105 1110 1115 1120

Tyr Met Thr His Pro Ser Val Gln Ala His His Gln Gln Gln Leu
1125 1130 1135

That which is claimed is:

1. Isolated nucleic acid encoding a mammalian SCA2 polypeptide.

2. Isolated nucleic acid according to claim 1, wherein said nucleic acid comprises DNA.

3. DNA according to claim 2, wherein said DNA is a cDNA.

4. DNA according to claim 2, wherein said DNA encodes at least about 10 contiguous amino acids set forth in SEQ ID NO:3, or SEQ ID NO:5.

5. DNA according to claim 2, wherein said DNA hybridizes under high stringency conditions to the SCA2 coding portion of nucleotides 1 - 516 of SEQ ID NO:1 or nucleotides 163-4098 of SEQ ID NO:2 , or nucleotides 50-3454 of SEQ ID NO:4.

6. DNA according to claim 2, wherein said DNA has substantially the same nucleotide sequence as the SCA2 coding portion set forth in SEQ ID NO:2, or SEQ ID NO:4.

7. A vector comprising DNA according to claim 2.

8. A host cell containing a vector according to claim 7, wherein said cell is a procaryotic cell or a eucaryotic cell.

9. A host cell according to claim 8, wherein said cell expresses a functional SCA2 protein.

10. An oligonucleotide comprising at least 15 nucleotides capable of specifically hybridizing with a sequence of nucleic acids of the nucleotide sequence set forth in SEQ ID NO:2, or SEQ ID NO:4.

11. An oligonucleotide according to claim 10, wherein said oligonucleotide is labeled with a detectable marker.

12. A kit for detecting mutations and in chromosome 12 at the SCA2 locus in 12q24.1 comprising at least one oligonucleotide according to claim 10.

13. Isolated mRNA complementary to DNA according to claim 2.

14. An oligonucleotide composition comprising chemical analogues of the nucleic acid of claim 2 operatively linked to a promoter of RNA transcription.

15. An antisense oligonucleotide capable of specifically binding to and inhibiting the translation of mRNA according to claim 13.

16. Isolated SCA2 polypeptide, or fragments thereof, and functional equivalents thereof.

17. Isolated SCA2 polypeptide according to claim 16, wherein said polypeptide comprises substantially the same amino acid sequence as that set forth in SEQ ID NO:3, amino acids 1-165 or amino acids 188-1312 of SEQ ID NO:3, or substantially the same amino acid sequence as that set forth in SEQ ID NO:5.

18. Isolated SCA2 polypeptide according to claim 16, wherein said polypeptide has the same amino acid sequence as that set forth in SEQ ID NO:3, or at least amino acids 1-165 or amino acids 188-1312 of SEQ ID NO:3, or in SEQ ID NO:5.

19. Isolated SCA2 polypeptide according to claim 16, wherein said polypeptide is encoded by a nucleotide sequence that is substantially the same nucleotide sequence as that set forth in SEQ ID NO:2, nucleotides 163-4098 of SEQ ID NO:2, SEQ ID NO:4, or nucleotides 50-3454 of SEQ ID NO:4.

20. Isolated SCA2 polypeptide according to claim 16, wherein said polypeptide is encoded by at least nucleotides 163-4098 set forth in SEQ ID NO:2, or at least nucleotides 50-3454 of SEQ ID NO:4.

21. An SCA2 polypeptide expressed recombinantly in a host cell.

22. An SCA2 polypeptide according to claim 21, wherein said polypeptide is encoded by a nucleotide sequence that is substantially the same as at least nucleotides 163-4098 set forth in SEQ ID NO:2, or at least nucleotides 50-3454 of SEQ ID NO:4.

23. An SCA2 polypeptide according to claim 21, wherein said polypeptide is encoded by at least nucleotides 163-4098 set forth in SEQ ID NO:2, or at least nucleotides 50-3454 of SEQ ID NO:4.

24. An antibody that specifically binds to a determinant on a SCA2 polypeptide according to claim 16, or active fragment thereof.

25. An antibody according to claim 24, wherein said antibody is a monoclonal antibody.

26. An antibody according to claim 24, wherein said antibody is a polyclonal antibody.

27. A composition comprising an amount of the antisense oligonucleotide according to claim 13 effective to modulate expression of a human SCA2 polypeptide and an acceptable hydrophobic carrier capable of passing through a cell membrane.

28. A composition according to claim 27, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

29. A composition according to claim 28, wherein said substance is a ribozyme.

30. A composition comprising an amount of an antibody according to claim 24 effective to block function of the SCA2 protein or to block interaction of the SCA2 protein with other proteins or ligands.

31. A transgenic nonhuman mammal expressing DNA encoding a SCA2 polypeptide according to claim 2.

32. A transgenic nonhuman mammal according to claim 31, wherein said DNA encoding said polypeptide has been mutated as to be incapable of normal polypeptide activity, and wherein the polypeptide so expressed is not native SCA2 polypeptide.

33. A transgenic nonhuman mammal, the genome of which comprising antisense DNA complementary to DNA encoding a SCA2 polypeptide according to claim 2, wherein said antisense DNA is transcribed into antisense mRNA complementary to mRNA encoding a human SCA2 polypeptide.

34. A transgenic nonhuman mammal according to claim 31, wherein said DNA is operatively linked to an inducible promoter.

35. A transgenic nonhuman mammal according to claim 31, wherein said DNA is operatively linked to tissue specific regulatory elements.

36. A transgenic nonhuman mammal according to claim 31, wherein the transgenic nonhuman mammal is a mouse.

37. A method for identifying nucleic acids encoding a human SCA2 protein, said method comprising:

contacting a sample containing nucleic acids with a probe according to claim 11, wherein said contacting is effected under high stringency hybridization conditions, and identifying compounds which hybridize thereto.

38. A method for identifying compound(s) which bind to a human SCA2 polypeptide, said method comprising contacting cells according to claim 9 with said compound(s) and identifying compounds which bind thereto.

39. A method for detecting the presence of a human SCA2 polypeptide, said method comprising contacting a test sample with an antibody according to claim 24, detecting the presence of an antibody-SCA2 complex, and therefor detecting the presence of a human SCA2 polypeptide in said test sample.

40. Single strand DNA primers for amplification diagnosis of SCA2, wherein said primers comprise a nucleic acid sequence derived from the nucleic acid according to claim 1 set forth as SEQ ID NO:2, or SEQ ID NO:4.

41. A method for diagnosing spinocerebellar Ataxia Type 2, said method comprising:

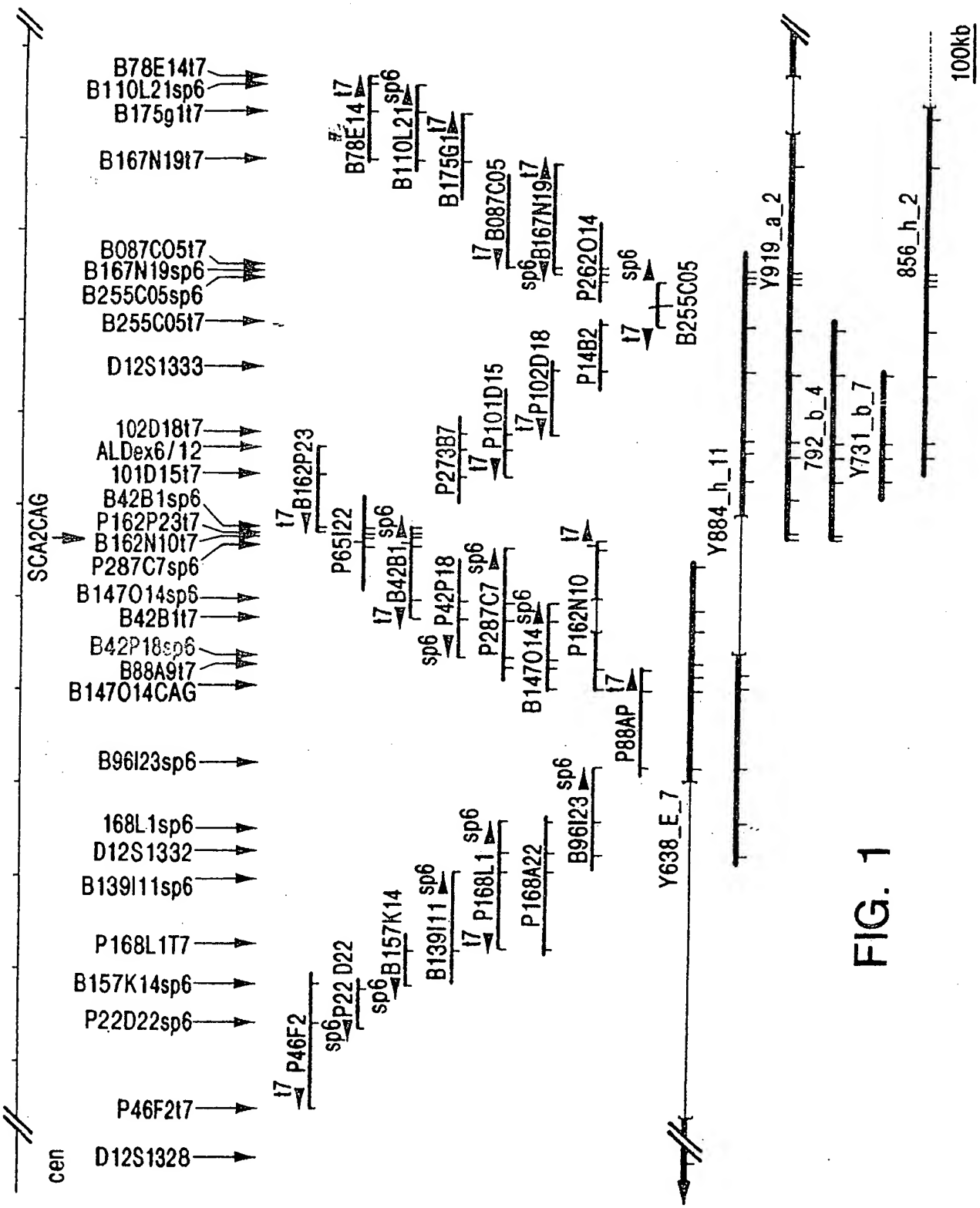
detecting, in said subject, a genomic or transcribed mRNA sequence having an expanded CAG repeat at a location corresponding to between nucleotides 657 and 724 of SEQ ID NO:2.

42. A method for diagnosing spinocerebellar Ataxia Type 2, said method comprising:

a) contacting nucleic acid obtained from a subject suspected of having SCA2 with primers that amplify at least a nucleic acid fragment of SEQ ID NO:2 containing nucleotides 658-723 of SEQ ID NO:2, under conditions suitable to form a detectable amplification product; and

b) detecting an amplification product containing substantially expanded CAG repeats above normal, whereby said detection indicates that said subject has SCA2.

43. A diagnostic kit comprising at least one oligonucleotide according to claim 10 contained in a packaging material.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B175g1t7
B167N19t7
B087C05t7
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D12S1333
102D18t7
ALDex6/12
101D15t7
B42B1sp6
P162P23t7
B162N10t7
P287C7sp6
B147O14sp6
B42B1t7
B42P18sp6
B88A9t7
B147O14CAG
B96I23sp6
168L1sp6
D12S1332
B139I11sp6
P168L1T7
B157K14sp6
P22D22sp6
P46F2t7
D12S1328
cen
SCA2CAG
P46F2
P22 D22
B157K14
B139I11
P168L1
P168A22
B96I23
P88AP
P162N10
B147O14
P287C7
P42P18
B42B1
P65I22
B162P23
P273B7
P101D15
P102D18
P14B2
P262O14
B255C05
B087C05
B167N19
B175G1
B110L21
B78E14
Y638_E_7
Y884_h_11
Y919_a_2
792_b_4
Y731_b_7
856_h_2
100kb

FIG. 1

```
  1  TTGGTAGCAACGGAAACGGCGGCGGCGCGTTTCGGCCCGGCTCCCGGCGGCTCCTTGGTC

 61  TCGGCGGGCCTCCCCGCCCCTTCGTCGTCGTCCTTCTCCCCCTCGCCAGCCCGGGCGCCC

121  CTCCGGCCGCGCCAACCCGCGCCTCCCCGCTCGGCGCCCGTGCGTCCCCGCCGCGTTCCG

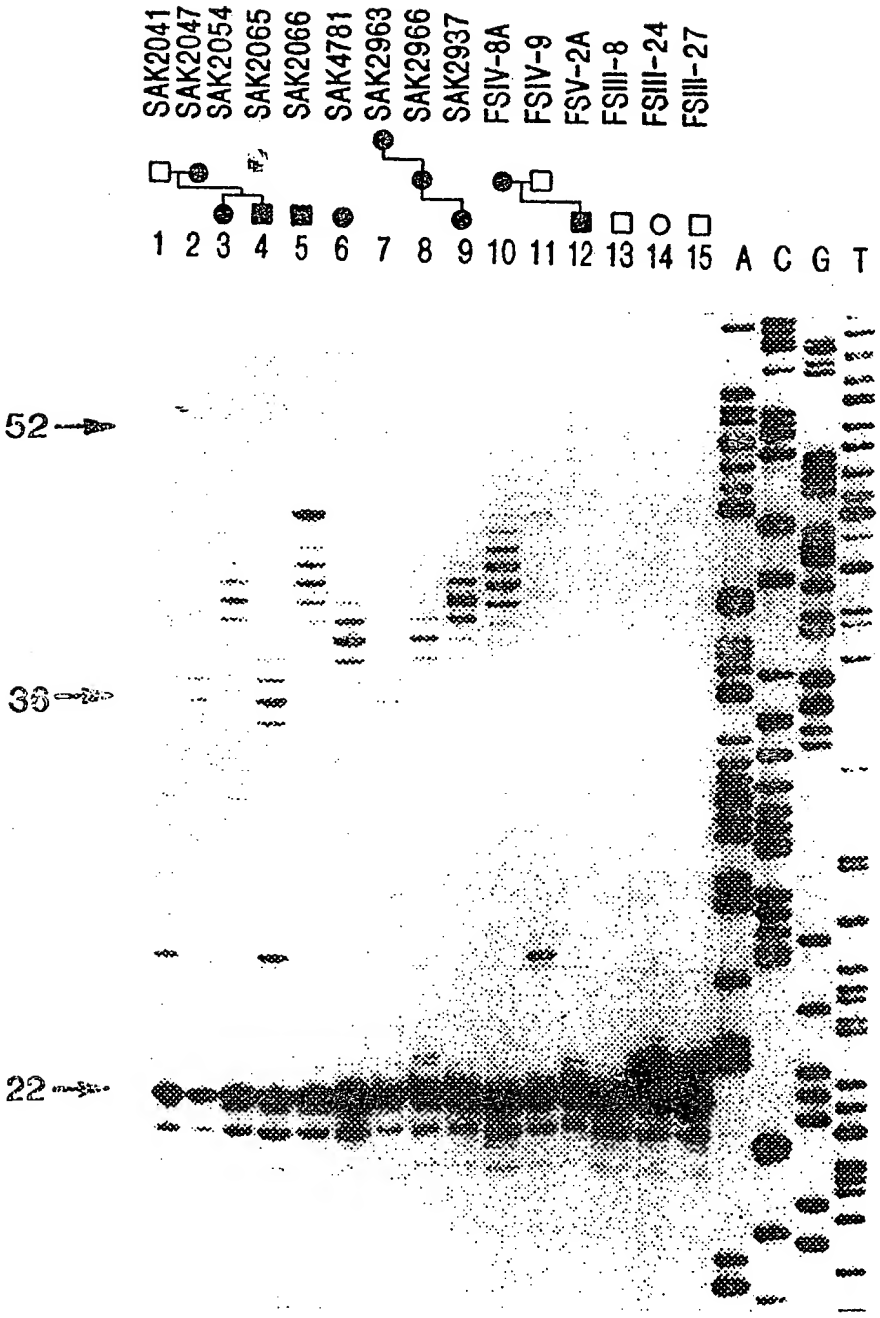
181  GCGTCTCCTTGGCGCGCCCGGCTCCCGGCTGTCCCCGCCCGGCGTGCGAGCCGGTGTATG
          SCA2-A
241  GGCCCCTCACCATGTCGCTGAAGCCCCAGCAGCAGCAGCAGCAGCAGCAGCAACAGCAGC
                                                          SCA2-B
301  AGCAGCAACAGCAGCAGCAGCAGCAGCAGCAGCCGCCGCCCGCGGCTGCCAATGTCCGCA

361  AGCCCGGCGGCAGCGGCCTTCTAGCGTCGCCCGCCGCCGCGCCTTCGCCGTCCTCGTCCT

421  CGGTCTCCTCGTCCTCGGCCACGGCTCCCTCCTCGGTGGTCGCGGCGACCTCCGGCGGCG

481  GGAGGCCCGGCCTGGGCAG GTGGGTGTCGGCACCCC
```

FIG. 2


SAK2041
SAK2047
SAK2054
SAK2065
SAK2066
SAK4781
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SAK2937
FSIV-8A
FSIV-9
FSV-2A
FSIII-8
FSIII-24
FSIII-27
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 A C G T
52
36
22

FIG. 3

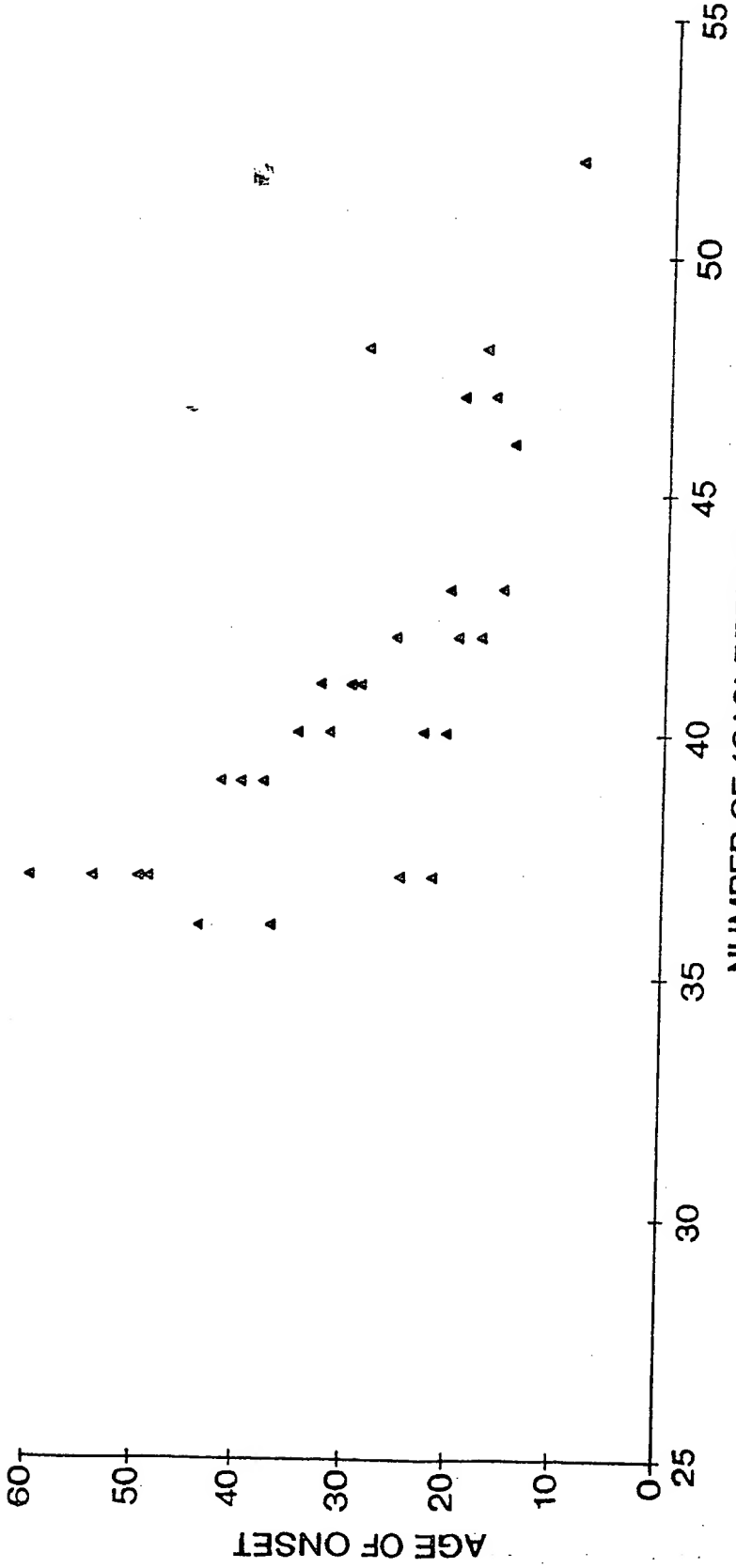

AGE OF ONSET
0
10
20
30
40
50
60
NUMBER OF (CAG) REPEATS
25
30
35
40
45
50
55

FIG. 4

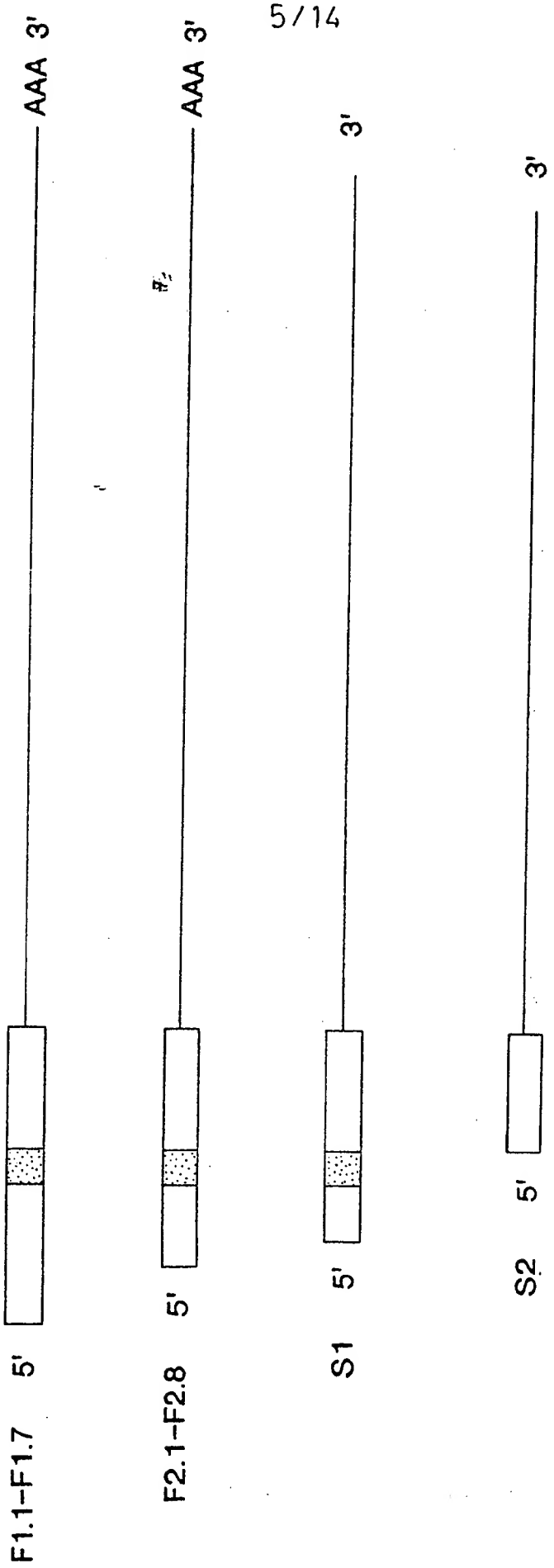


FIG. 5

```
  1  ACCCCCGAGAAAGCAACCCAGCGCGCCGCCCGCTCCTCACGTGTCCCTCCCGGCCCCGGG   60
 61  GCCACCTCACGTTCTGCTTCCGTCTGACCCCTCCGACTTCCGGTAAAGAGTCCCTATCCG  120
                                 *
121  CACCTCCGCTCCCACCCGGCGCCTCGGCGCGCCCGCCCTCCGATGCGCTCAGCGGCCGCA  180
  1                                                 M  R  S  A  A  A    6
181  GCTCCTCGGAGTCCCGCGGTGGCCACCGAGTCTCGCCGCTTCGCCGCAGCCAGGTGGCCC  240
  7  A  P  R  S  P  A  V  A  T  E  S  R  R  F  A  A  A  R  W  P     26
241  GGGTGGCGCTCGCTCCAGCGGCCGGCGCGGCGGAGCGGGCGGGGCGGCGGTGGCGCGGCC  300
 27  G  W  R  S  L  Q  R  P  A  R  R  S  G  R  G  G  G  G  A  A     46
301  CCGGGACCGTATCCCTCCGCCGCCCCTCCCCCGCCCGGCCCCGGCCCCCCTCCCTCCCGG  360
 47  P  G  P  Y  P  S  A  A  P  P  P  P  G  P  G  P  P  P  S  R     66
361  CAGAGCTCGCCTCCCTCCGCCTCAGACTGTTTTGGTAGCAACGGCAACGGCGGCGGCGCG  420
 67  Q  S  S  P  P  S  A  S  D  C  F  G  S  N  G  N  G  G  G  A     86
421  TTTCGGCCCGGCTCCCGGCGGCTCCTTGGTCTCGGCGGGCCTCCCCGCCCCTTCGTCGTC  480
 87  F  R  P  G  S  R  R  L  L  G  L  G  G  P  P  R  P  F  V  V    106
481  GTCCTTCTCCCCCTCGCCAGCCCGGGCGCCCCTCCGGCCGCGCCAACCCGCGCCTCCCCG  540
107  V  L  L  P  L  A  S  P  G  A  P  P  A  A  P  T  R  A  S  P    126
541  CTCGGCGCCCGTGCGTCCCCGCCGCGTTCCGGCGTCTCCTTGGCGCGCCCGGCTCCCGGC  600
127  L  G  A  R  A  S  P  P  R  S  G  V  S  L  A  R  P  A  P  G    146
                                    SCA2-A
601  TGTCCCCGCCCGGCGTGCGAGCCGGTGTATGGGCCCCTCACCATGTCGCTGAAGCCCCAG  660
147  C  P  R  P  A  C  E  P  V  Y  G  P  L  T  M  S  L  K  P  Q    166
661  CAGCAGCAGCAGCAGCAGCAACAGCAGCAGCAGCAACAGCAGCAGCAGCAGCAGCAG    720
167  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q    186
```

FIG. 6A

SCA2-B

```
721  CAGCCGCCGCCCGCGGCTGCCAATGTCCGCAAGCCCGGCGGCAGCGGCCTTCTAGCGTCG  780
187  Q  P  P  P  A  A  A  N  V  R  K  P  G  G  S  G  L  L  A  S   206
781  CCCGCCGCCGCGCCTTCGCCGTCCTCGTCCTCGGTCTCCTCGTCCTCGGCCACGGCTCCC  840
207  P  A  A  A  P  S  P  S  S  S  S  V  S  S  S  S  A  T  A  P   226
841  TCCTCGGTGGTCGCGGCGACCTCCGGCGGCGGGAGGCCCGGCCTGGGCAGAGGTCGAAAC  900
227  S  S  V  V  A  A  T  S  G  G  G  R  P  G  L  G  R  G  R  N   246
901  AGTAACAAAGGACTGCCTCAGTCTACGATTTCTTTTGATGGAATCTATGCAAATATGAGG  960
247  S  N  K  G  L  P  Q  S  T  I  S  F  D  G  I  Y  A  N  M  R   266
961  ATGGTTCATATACTTACATCAGTTGTTGGCTCCAAATGTGAAGTACAAGTGAAAAATGGA  1020
267  M  V  H  I  L  T  S  V  V  G  S  K  C  E  V  Q  V  K  N  G   286
```

SCA2-14B

```
1021 GGTATATATGAAGGAGTTTTTAAAACTTACAGTCCGAAGTGTGATTTGGTACTTGATGCC  1080
287  G  I  Y  E  G  V  F  K  T  Y  S  P  K  C  D  L  V  L  D  A   306
1081 GCACATGAGAAAAGTACAGAATCCAGTTCGGGGCCGAAACGTGAAGAAATAATGGAGAGT  1140
307  A  H  E  K  S  T  E  S  S  S  G  P  K  R  E  E  I  M  E  S   326
1141 ATTTTGTTCAAATGTTCAGACTTTGTTGTGGTACAGTTTAAAGATATGGACTCCAGTTAT  1200
327  I  L  F  K  C  S  D  F  V  V  V  Q  F  K  D  M  D  S  S  Y   346
1201 GCAAAAAGAGATGCTTTTACTGACTCTGCTATCAGTGCTAAAGTGAATGGCGAACACAAA  1260
347  A  K  R  D  A  F  T  D  S  A  I  S  A  K  V  N  G  E  H  K   366
1261 GAGAAGGACCTGGAGCCCTGGGATGCAGGTGAACTCACAGCCAATGAGGAACTTGAGGCT  1320
367  E  K  D  L  E  P  W  D  A  G  E  L  T  A  N  E  E  L  E  A   386
1321 TTGGAAAATGACGTATCTAATGGATGGGATCCCAATGATATGTTTCGATATAATGAAGAA  1380
387  L  E  N  D  V  S  N  G  W  D  P  N  D  M  F  R  Y  N  E  E   406
1381 AATTATGGTGTAGTGTCTACGTATGATAGCAGTTTATCTTCGTATACAGTGCCCTTAGAA  1440
407  N  Y  G  V  V  S  T  Y  D  S  S  L  S  S  Y  T  V  P  L  E   426
1441 AGAGATAACTCAGAAGAATTTTTAAAACGGGAAGCAAGGGCAAACCAGTTAGCAGAAGAA  1500
427  R  D  N  S  E  E  F  L  K  R  E  A  R  A  N  Q  L  A  E  E   446
```

FIG. 6B

```
1501  ATTGAGTCAAGTGCCCAGTACAAAGCTCGAGTGGCCCTGGAAAATGATGATAGGAGTGAG  1560
 447  I  E  S  S  A  Q  Y  K  A  R  V  A  L  E  N  D  D  R  S  E    466
1561  GAAGAAAAATACACAGCAGTTCAGAGAAATTCCAGTGAACGTGAGGGGCACAGCATAAAC  1620
 467  E  E  K  Y  T  A  V  Q  R  N  S  S  E  R  E  G  H  S  I  N    486
1621  ACTAGGGAAAATAAATATATTCCTCCTGGACAAAGAAATAGAGAAGTCATATCCTGGGGA  1680
 487  T  R  E  N  K  Y  I  P  P  G  Q  R  N  R  E  V  I  S  W  G    506
1681  AGTGGGAGACAGAATTCACCGCGTATGGGCCAGCCTGGATCGGGCTCCATGCCATCAAGA  1740
 507  S  G  R  Q  N  S  P  R  M  G  Q  P  G  S  G  S  M  P  S  R    526
1741  TCCACTTCTCACACTTCAGATTTCAACCCGAATTCTGGTTCAGACCAAAGAGTAGTTAAT  1800
 527  S  T  S  H  T  S  D  F  N  P  N  S  G  S  D  Q  R  V  V  N    546
1801  GGAGGTGTTCCCTGGCCATCGCCTTGCCCATCTCCTTCCTCTCGCCCACCTTCTCGCTAC  1860
 547  G  G  V  P  W  P  S  P  C  P  S  P  S  S  R  P  P  S  R  Y    566
1861  CAGTCAGGTCCCAACTCTCTTCCACCTCGGGCAGCCACCCCTACACGGCCGCCCTCCAGG  1920
 567  Q  S  G  P  N  S  L  P  P  R  A  A  T  P  T  R  P  P  S  R    586
1921  CCCCCCTCGCGGCCATCCAGACCCCCGTCTCACCCCTCTGCTCATGGTTCTCCAGCTCCT  1980
 587  P  P  S  R  P  S  R  P  P  S  H  P  S  A  H  G  S  P  A  P    606
1981  GTCTCTACTATGCCTAAACGCATGTCTTCAGAAGGGCCTCCAAGGATGTCCCCAAAGGCC  2040
 607  V  S  T  M  P  K  R  M  S  S  E  G  P  P  R  M  S  P  K  A    626
2041  CAGCGACATCCTCGAAATCACAGAGTTTCTGCTGGGAGGGGTTCCATATCCAGTGGCCTA  2100
 627  Q  R  H  P  R  N  H  R  V  S  A  G  R  G  S  I  S  S  G  L    646
2101  GAATTTGTATCCCACAACCCACCCAGTGAAGCAGCTACTCCTCCAGTAGCAAGGACCAGT  2160
 647  E  F  V  S  H  N  P  P  S  E  A  A  T  P  P  V  A  R  T  S    666
2161  CCCTCGGGGGGAACGTGGTCATCAGTGGTCAGTGGGGTTCCAAGATTATCCCCTAAAACT  2220
 667  P  S  G  G  T  W  S  S  V  V  S  G  V  P  R  L  S  P  K  T    686
2221  CATAGACCCAGGTCTCCCAGACAGAACAGTATTGGAAATACCCCCAGTGGGCCAGTTCTT  2280
 687  H  R  P  R  S  P  R  Q  N  S  I  G  N  T  P  S  G  P  V  L    706
```

FIG. 6C

```
3121  CCCATGCCAGTGAATCAAGCCAAGACATATAGAGCAGTACCAAATATGCCCCAACAGCGG  3180
 987  P  M  P  V  N  Q  A  K  T  Y  R  A  V  P  N  M  P  Q  Q  R   1006
3181  CAAGACCAGCATCATCAGAGTGCCATGATGCACCCAGCGTCAGCAGCGGGCCCACCGATT  3240
1007  Q  D  Q  H  H  Q  S  A  M  M  H  P  A  S  A  A  G  P  P  I   1026
3241  GCAGCCACCCCCACCAGCTTACTCCACGCAATATGTTGCCTACAGTCCTCAGCAGTTCCCA  3300
1027  A  A  T  P  P  A  Y  S  T  Q  Y  V  A  Y  S  P  Q  Q  F  P   1046
3301  AATCAGCCCCTTGTTCAGCATGTGCCACATTATCAGTCTCAGCATCCTCATGTCTATAGT  3360
1047  N  Q  P  L  V  Q  H  V  P  H  Y  Q  S  Q  H  P  H  V  Y  S   1066
3361  CCTGTAATACAGGGTAATGCTAGAATGATGGCACCACCAACACACGCCCAGCCTGGTTTA  3420
1067  P  V  I  Q  G  N  A  R  M  M  A  P  P  T  H  A  Q  P  G  L   1086
3421  GTATCTTCTTCAGCAACTCAGTACGGGGCTCATGAGCAGACGCATGCGATGTATGCATGT  3480
1087  V  S  S  S  A  T  Q  Y  G  A  H  E  Q  T  H  A  M  Y  A  C   1106
3481  CCCAAATTACCATACAACAAGGAGACAAGCCCTTCTTTCTACTTTGCCATTTCCACGGGC  3540
1107  P  K  L  P  Y  N  K  E  T  S  P  S  F  Y  F  A  I  S  T  G   1126
3541  TCCCTTGCTCAGCAGTATGCGCACCCTAACGCTACCCTGCACCCACATACTCCCACACCCT  3600
1127  S  L  A  Q  Q  Y  A  H  P  N  A  T  L  H  P  H  T  P  H  P   1146
3601  CAGCCTTCAGCTACCCCCACTGGACAGCAGCAAAGCCAACATGGTGGAAGTCATCCTGCA  3660
1147  Q  P  S  A  T  P  T  G  Q  Q  Q  S  Q  H  G  G  S  H  P  A   1166
3661  CCCAGTCCTGTTCAGCACCATCAGGCCGCCCAGGCTCTCCATCTGGCCAGTCCA        3720
```

FIG. 6E

```
4021  CCCATTCCAGTCTCGACAACAGCGCATTTCCCCTATATGACGCACCCTTCAGTACAAGCC   4080
1287  P  I  P  V  S  T  T  A  H  F  P  Y  M  T  H  P  S  V  Q  A     1306
4081  CACCACCAACAGCAGTTGTAAGGCTGCCCTGGAGGAACCGAAAGGCCAAATTCCCTCCTC   4140
1307  H  H  Q  Q  Q  L  *                                           1326
4141  CCTTCTACTGCTTCTACCAACTGGAAGCACAGAAAACTAGAATTTCATTTATTTTGTTTT   4200
4201  TAAAATATATATGTTGATTTCTTGTAACATCCAATAGGAATGCTAACAGTTCACTTGCAG   4260
4261  TGGAAGATACTTGGACCGAGTAGAGGCATTTAGGAACTTGGGGGCTATTCCATAATTCCA   4320
4321  TATGCTGTTTCAGAGTCCCGCAGGTACCCCAGCTCTGCTTGCCGAAACTGGAAGTTATTT   4380
4381  ATTTTTTAATAACCCTTGAAAGTCATGAACACATCAGCTAGCAAAAGAAGTAACAAGAGT   4440
4441  GATTCTTGCTGCTATTACTGCTAAAAAAAAAAAAAAAAAAA  4481
```

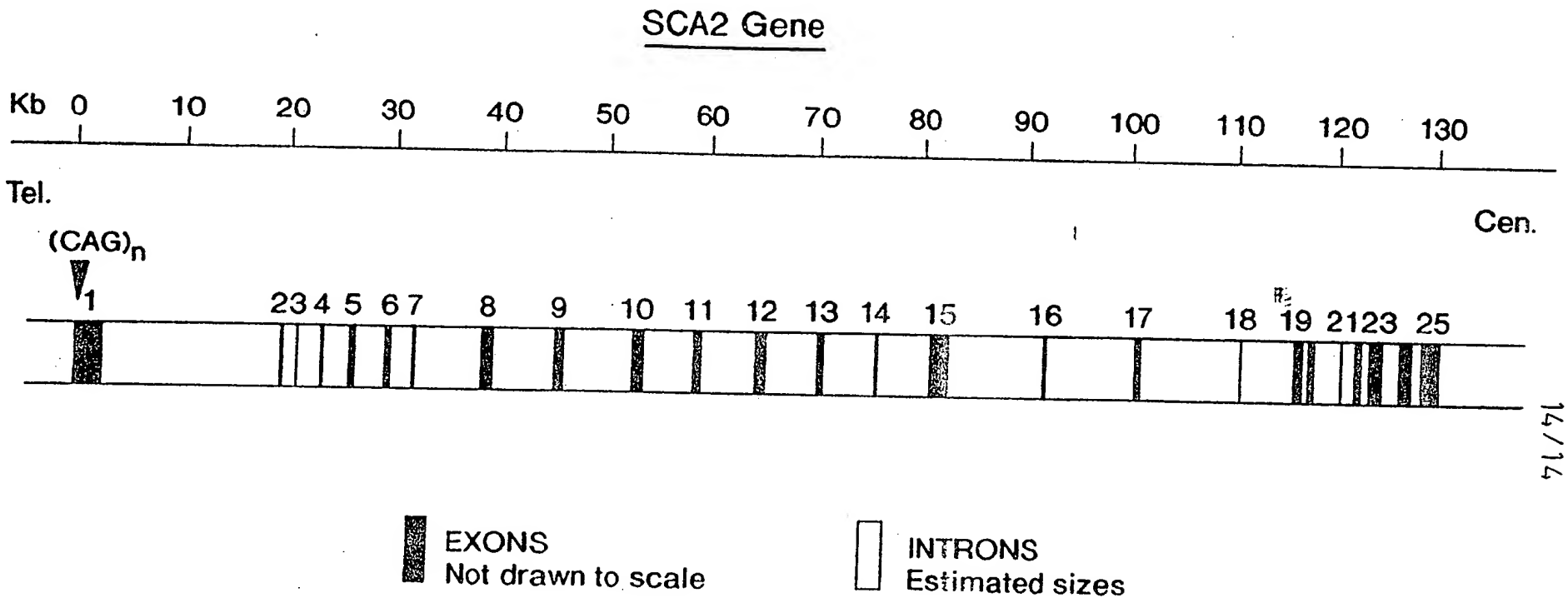
FIG. 6F

```
A2RP        LAVDAVHRKA SEPAGGPRRE DIVDTMVFKP SDVMLVHFRN VDFNYATKDK
Consensus   L--DA-H-K- -E---GP-RE -I-----FK- SD---V-F-- -D--YA--D-
```

FIG. 7A

```
Consensus   --
```

FIG. 7B



-Largest exon: exon 1, 928 bps; contains CAG repeat
-Largest intron: intron 1 with approximately 15 Kbps
-Smallest exon: exon 2, 37 bps

- Exon sizes:
  8<100 bps
  100 bps<12<200 bps
  200 bps< 4<400 bps
  400 bps< 1

- known intron sizes:
  intron 2 : 1.6 Kb
  intron 19: 0.3 Kb
  intron 22: 1.0 Kb
  intron 24: 1.6 Kb

FIG. 8

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 97/07725

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 C12N15/12 C12N5/10 C07K14/47 C07K16/18
C12Q1/68 G01N33/577 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 97 17445 A (CENTRE NAT RECH SCIENT ;INST NAT SANTE RECH MED (FR); TORA LAZSLO) 15 May 1997 see page 18, line 25 - page 21, line 14 ---	1-30, 37-43
X	NATURE, vol. 378, no. 6555, 23 November 1995, pages 403-406, XP002009617 TROTTIER Y ET AL: "POLYGLUTAMINE EXPANSION AS A PATHOLOGICAL EPITOPE IN HUNTINGTON'S DISEASE AND FOUR DOMINANT CEREBELLAR ATAXIAS" see the whole document ---	24,25,39

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 1997	16.09.97

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INTERNATIONAL SEARCH REPORT

Inter mal Application No
PCT/US 97/07725

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 95 01437 A (UNIV MINNESOTA) 12 January 1995 see the whole document	1-43
P,X	HUMAN MOLECULAR GENETICS, vol. 5, no. 12, December 1996, pages 1887-1892, XP002027564 STEVANIN G ET AL: "SCREENING FOR PROTEINS WITH POLYGLUTAMINE EXPANSIONS IN AUTOSOMAL DOMINANT CEREBELLAR ATAXIAS" see the whole document	24,25,39
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INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/US 97/07725

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		EP 0707647 A	24-04-96
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